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INTEGUMENTARY PHOTOSENSITIVITY IN A MARINE FISH, EPINEPHELUS STRIATUS BLOCH

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CONTENTS

I. INTRODUCTION

A. Literature

Experimental evidence, although it is not extensive, has consistently indicated that the integuments of fresh-water fishes are photosensitive, but that those of the strictly marine members in this class, on the other hand, are insensitive to light. Parker ('09) states that the follow-

ing marine fishes—Mustelus canis, Anguilla chryspa, Stenotomus chrysops, Tautogolabrus adspersus, Tautoga onitis, Chilomycterus schoepfi, Opsanus tau and Microgradus tomcod—possess integuments which are unresponsive to photic stimulation. He concludes, therefore, that cutaneous photosensitivity may be a characteristic of fresh-water fishes, as phosphorescence is of certain marine organisms.

But in the course of certain experiments upon the hamlet (Epinephelus striatus), I discovered, contrary to this theory, that both normal and blinded fishes are negatively phototropic. Their reactions closely resembled those of the fresh-water fishes which have already been described by several authors. Eigenmann ('00) has shown that the blind fish Amblyopsis is negatively phototropic; Parker ('05) found the common brook lamprey, Ammocoetes, to be negatively phototropic and also photodynamic; and Payne ('07), who studied Amblyopsis, states that this fish is negatively phototropic and photodynamic regardless of the direction of the rays of light and also that its sensitivity to light decreases with age.

Fundulus, however, is reported to be an exception to the theory of universal cutaneous photosensitivity among the non-marine fishes, since Long (see Parker '05) and Parker ('09, p. 77) were unable to stimulate its skin with light. It might, however, be urged in explanation of this apparent exception that Fundulus is adapted to life in salt water (as well as in fresh) and simply shares with other marine fishes their characteristic insensitivity to light.

It is therefore of considerable interest that at length a marine fish with no known proclivities for life in fresh water has been found in which there is a marked photic sensitivity. Though only a single case, this may well cause one to hesitate in adopting the view that there is any fundamental and constant difference in this respect between the fishes of salt and of fresh water. It will perhaps also suggest that photosensitivity of the skin of lower verebrates is a capacity which has been independently acquired (or retained?) by remotely related forms.

B. Purpose of experiments

In view of the interest attaching to the question and the importance of a critical examination of these responses, further experiments were at once undertaken. They aimed, first, to determine more carefully the positive or negative character of the phototropism observed; secondly, to discover any evidence of photodynamic responses; and,

thirdly, to ascertain, if possible, any variation in the degree of sensitivity manifested by various regions of the integument. The probable location of the photo-receptive end organs, whether they lie in the integument or, as Payne ('07) has suggested, within the spinal cord, was also considered. During the course of the work several incidental problems arose, principally the questions of the physico-chemical nature of the effect of light upon the sense organs and the phenomenon of their exhaustion. Some suggestive data were obtained on these points but it is not the purpose of this paper to attempt their description and explanation in detail.

C. Material and methods

The hamlet, or grouper, proved to be a very favorable animal for these experiments because it is unusually hardy and recovers quickly and completely from severe operations, apparently manifesting no abnormalities of behavior.[1] In order to determine the characteristic reactions of the fish, normal individuals were studied first; but all subsequent investigations were conducted with hamlets whose eyes had been enucleated at least twenty-four hours previous to the time of the experiment. All fishes were kept in a large spawning pool which was supplied with a stream of fresh sea-water. It was usually necessary to prepare fresh individuals every second or third day, because fishes kept for a longer time manifested variations in response which were probably caused by hunger, fatigue and the loss of eyes.[2] In order that exhaustion of the photoreceptors might not modify the responses, each fish was used for only one, or at most two, sets of experiments embracing from six to twelve tests each.

The specimen under investigation—all experiments were conducted at night—was placed in an aquarium whose sides were of glass and whose top, bottom and ends were of an opaque material. After the fish had become habituated to the new environment its integument was illuminated with light from electric lamps of various moderate inten-

[1] This characteristic can hardly be exaggerated. The fishes endure the severest of operations, either on the central nervous system or on the body, with remarkable resistance.

[2] The loss of eyes seems to cause an increase in the activity of some cutaneous faculties, i.e., sensitivity to tactile and rheotropic stimuli; but the phototropic responses, on the other hand, are consistently retarded. It is likewise almost impossible to induce blinded fishes to eat and their olfactory responses are more or less retarded. These facts are interesting as showing something of the rôle of optic stimuli in the life of the normal fish.

sities. These were placed within a small wooden reflector-box which had a small hole in one end. The box was lined with tin for the purpose of increasing the intensity of the illumination. It is to be regretted that no means was available for testing the exact intensity of the light;[3] but for the study of variations in the sensitivity in different regions of the integument and of photodynamic responses, relative intensities, such as are obtained from wattages of various magnitudes, are sufficiently precise. The illumination of the fish, however, was less intense than the indicated wattage of the lamp, because a certain

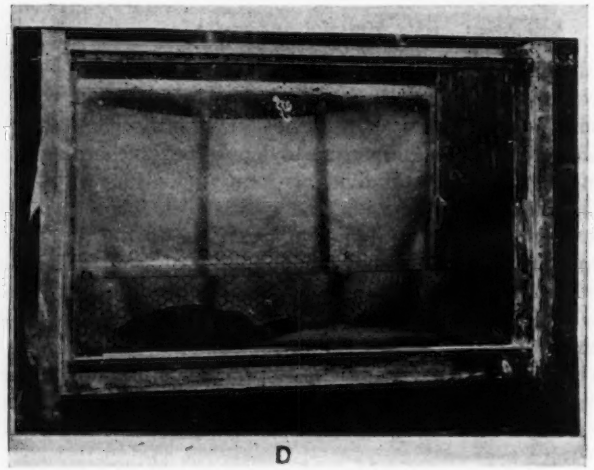

Figure of the aquarium used in testing fishes for photosensitivity

amount of light was cut off by the edges of the reflector-box and by the water[4] between the fish and the light.

These experiments were conducted in an aquarium which has already been figured and described elsewhere (Jordan '17); the figure is repro-

[3] The candle power of mazda electric light bulbs is approximately 1 per watt; but, as the candle power was not measured in the following experiments, wattages instead of candle powers will be used to express the intensity of illumination.

[4] Temperature changes are negligible, since a thermometer held in the position occupied by the fish was unaffected by the light. There was usually about 6 to 8 inches of water between the light, which was held against the glass of the aquarium, and the fish.

duced here. There were no currents in the tank during the experiments with light, but the sea-water was renewed at frequent intervals. For the purpose of determining the positive or negative character of the hamlet's phototropism, one-half of the water in the tank, which was approximately 15 x 15 x 12 inches in size, was darkened by an opaque black screen; the other half, containing the fish, being fully illuminated by electric lamps of various intensities. Thus were created so-called light and dark fields. If a fish, manifesting the fin and body movements characteristic of photic stimulation,[5] remained in the lighted zone, it was regarded as positively phototropic, whereas swimming into the darkened portion of the tank was taken as evidence of negative phototropism.

In the detection of differences in the degree of sensitivity of various regions of the integument, as well as for securing additional evidence concerning the positive or negative character of the phototropism, the light was localized, rather imperfectly, upon different regions of the body by means of absorbing screens of black pasteboard and mirrors which served as reflectors. It was difficult to procure a delicate local stimulation by these methods and the areas described are large and overlap each other more or less.

The writer is deeply grateful for the assistance which Dr. E. L. Mark and Dr. W. J. Crozier have rendered in connection with this work, and also for the privilege of study at the Bermuda Biological Station. He also wishes to thank the trustees of the Humboldt Fund for financial aid.

II. DESCRIPTION OF EXPERIMENTS

When a normal fish was placed in the testing aquarium, it swam about vigorously for a few moments, bumping the walls with its head or tail. This uneasiness soon disappeared, however, and it settled to the bottom, where, unless disturbed, it remained indefinitely. If now its body were sufficiently illuminated, it soon began to swim about, and usually did not resume a resting position until the light was removed or until it reached the dark end of the aquarium. The criterion of stimulation adopted for these experiments was to start the fish in this way from a position of rest and then, by cutting off the light, to cause a resumption of the original attitude.

[5] The fishes when first illuminated often twitched their dorsal fins rapidly and changed position slightly. No increase in the rate of respiration was noted and the fin movements were but little changed in rate. Ultimately illumination led to more or less continuous swimming about.

A. Reactions of normal fishes

1. To light and dark fields. Light- and dark-field experiments, in which the half of the aquarium containing the fish was illuminated, were often repeated and almost invariably a decided negative reaction was observed. The fishes with few exceptions swam away from the lighted end of the aquarium when a 60-watt light was used as a stimulus. This reaction occurred in approximately 60 seconds, occasionally after a much longer time. Then after a variable period of relative quiescence in the dark, the fish often returned to the lighted zone for an interval, shorter, usually, than the sojourn in the shaded end. Illumination usually caused uneasiness, the fish being constantly in motion. In many instances this swimming from light to dark and back to light was repeated several times; but until the photoreceptors became exhausted, successive illuminations seldom failed to cause a retreat to the dark—a very definite negative reaction.

2. To regional stimulation. Negative responses were also obtained in nearly all cases when a portion only of the integument was illuminated by a 100-watt electric light. By testing different areas of the skin separately, the other regions being in comparative dark, it was found that various areas manifested this sensitivity in different degrees. The times of exposure required to produce evidences of stimulation on certain general areas of the body were as follows: Head and eyes, 1–5 (average 3) seconds; posterior end of the body, 4–17 (av. 7.6) seconds; and mid-trunk 10–20 (av. 12.5) seconds. In these experiments the whole of the head, the body posterior to the anus and the region between the pectoral fin and anus, were successively, and more or less exclusively, stimulated by the localized light. Each fish was tested several times in rapid succession; though some reactions were inexplicably variable in time, there was on the whole a noticeable uniformity in the duration of illumination which was necessary to produce a negative response. For instance, among the records of the reaction times when the stimulus was applied to the head region are many data which are as uniform as the following: 4, 3, 2, 1, 3, 2 seconds. Sometimes the stimulation required was much longer, 60 seconds being the greatest. But as a rule the reactions to localized stimulation were consistent and precise and the failures to respond were few.

Normal hamlets, then, are negatively phototropic when either the whole, or a considerable part, only, of their integuments are stimulated by electric light. Some areas of the skin, too, usually manifest a

greater degree of sensitivity than others. This susceptibility to photic stimulation decreases in the following order: head > tail > mid-body regions.

B. Reactions of blinded fishes

During the experiments on regional sensitivity, the eyes of the normal fishes employed were usually in comparative darkness. It is improbable, therefore, that they could have been sufficiently illuminated by diffused light to cause a reaction, even though their partial illumination may have had some influence on it. However, in order to determine whether the integument or the eyes were chiefly concerned in such responses as have been described, similar experiments were repeated on blinded fishes. The behavior of such "physiological" individuals when first placed in the testing-tank was very similar to that of the normal animals. Their period of preliminary uneasiness, however, was slightly longer and they more frequently collided with the walls of the aquarium, since at first they apparently had no idea of the dimensions of their confines.

1. To light and dark fields. A single light-field and dark-field experiment will be described in detail, in order to show the nature of the response of blinded fishes to light; there are exhibited several indications of stimulation which precede the true negative response—the swimming to the darkened end of the aquarium. When a hamlet whose eyes have been enucleated finds itself in that half of the tank which is lighted by a 60-watt lamp, its first visible reaction, the raising of the dorsal fin, occurs in 24 seconds. A few seconds later its chromatophores darken.[6] Next it swings its tail toward the light, and finally, after 75 seconds, swims to the darkened half of the aquarium, where it comes to rest. This position is retained for about 3 minutes, whereupon the fish often swims back, more or less completely, into the lighted zone. After a period of relative uneasiness there, which is usually shorter than the interval of quiet in the dark, the negative reaction is repeated.

Often in such experiments the attempt to return to the light is stopped when the head first becomes illuminated. This momentary hesitation is usually followed by a retreat to the darkened end. Some of the fishes when their heads entered the light, hesitated, then reversed ends, and finally backed part way into the light, as if to test

[6] Light causes the region of the integument which is illuminated to become darker in color. Sunlight also darkens the color of the skin and shading lightens it.

the stimulation by means of the tail.[7] They then usually returned to the shadow, but sometimes continued toward the light until they were completely illuminated. This alternation of positions is almost identical with that which the normal fishes executed; but in the latter I did not notice any backing into the light.

Such behavior is typical of the several individuals which were tested in this manner. With few exceptions they all swam away from the light; thus being, as the normal fishes are, negatively phototropic to general illumination. Their reaction time, though somewhat variable, averages slightly longer than that of normal fishes, being 70 seconds for the stimulus of a 60-watt lamp at a distance of about 30 centimeters. A 100-watt light causes the same reaction in less time.

2. To regional stimulation. The integuments of blinded fishes also were explored with a localized light in order to determine whether the variations in regional sensitivity which the normal fishes exhibited would persist after the enucleation of both eyes. The apparatus used and the regions tested in these experiments were the same as those which have already been described in the account of the reactions of normal groupers.

They revealed a regional sensitivity nearly the same as that of the normal fishes; the head being the most sensitive, the tail intermediate and the mid-body least sensitive. But the average time of reaction to the same intensity of illumination was, for each region, longer than that of the normal fishes. A few experiments to determine this relation showed that on the average[8] the period of exposure necessary to produce stimulation is increased by the removal of the eyes as follows: in the head region, from 3 seconds to 8.4 seconds, or 180 per cent; in the mid-body region, from 12.5 seconds to 22.5 seconds, or 80 per cent; and in the tail region from 7.6 seconds to 11.5 seconds, or 51 per cent.

This retardation, it is to be observed, varies, but not proportionally

[7] It is interesting in this connection to note that the hamlet also backs into a current (Jordan '17) and swims backward into the cavities of its natural environment. It also uses the tail frequently as a tactile organ. The posterior part of the body may, therefore, have an important sensory function. Cutaneous sensitivity to most other stimuli (tactile, rhetropic, chemical) is very similar in its distribution to that to light. A somewhat similar case is one described by Parker ('05, p. 418). He states that the region of Ammocoetes which is most sensitive to light is its tail, and he coördinates this fact with the burrowing habit of the fish.

[8] There were, however, as usual, a few erratic cases in which the reaction time of the mid-body region was shorter than that of the tail, and others in which the reaction times of the three regions were equal.

to the sensitivity of the three regions, the order of the retardation being: head, mid-body, tail; that of sensitivity in both normal and blinded fishes: head, tail, mid-body. The explanation of this order of retardation is doubtless to be sought in the fact that it was impossible completely to screen the head when the mid-body and tail regions were exposed to light, with the result that the head (and consequently the eyes) were more highly stimulated by mid-body than by tail illumination.

The reaction times of the integument of normal and blinded fishes in different regions can not be regarded as wholly satisfactory, for the reason that, as above stated, the illumination of each region involved a greater or less illumination of the other regions. While, therefore, they show clearly which is the most and which the least sensitive area, they may not accurately represent the degree of sensitivity.

By way of summary, then, it may be said: first, that the integument of Epinephelus striatus exhibits a negative photosensitivity, which is independent of the eyes; secondly, that it also shows, fairly constantly, three regions of the integument which manifest this sensitivity in different degree as follows: head > tail > mid-body.

3. Photodynamic nature of the reponses. As noticed in most of the preceding experiments, the quickness of the response depends rather closely upon the intensity of stimulation. An increase in the wattage usually decreased the required period of stimulation and *vice versa*. This suggests that the responses may be photodynamic in nature.

Another set of experiments was, accordingly, undertaken for the purpose of testing the validity of this assumption. To do this the same local region, or the skin of one whole side, was stimulated successively with different intensities of light and the time of reaction carefully noted. In order to minimize the error which might be caused by exhaustion, care was taken to use alternately high and low intensities and especially to allow ample intervals for recovery. The results, some of which are given further on, substantiate the conclusions derived from the earlier observations, establishing the fact that the reactions of the hamlet to photic stimulation of the integument are dynamic in character.

The period of illumination required to produce a response in these experiments was found (as the result of about twenty-five tests at each wattage) to be for 100-,60- and 40-watt lamps 24, 35, and 53 seconds, respectively. In other words a decrease of 40 per cent from the highest intensity of the light (from 100 to 60 watts) resulted in an increase of about 45 per cent in the reaction time (from 24 to 35 seconds), and a 60 per cent decrease in the brightness of illumination (from 100 to 40

watts) increased the reaction time about 120 per cent (24 to 53 seconds). This rough inverse ratio between time and intensity, though based on methods not rigidly exact, conforms fairly well to the Bunsen-Roscoe law of photochemistry, viz., that the products of reaction time and intensity of stimulation are constant for any effective illumination. In my experiments upon the whole of one side the products for 100-, 60-, and 40-watt lights were 2400, 2100 and 2120, respectively. This conformity to the Bunsen-Roscoe law may, perhaps, be taken to indicate that photic stimulation of the integument of Epinephelus is photochemical in nature, as Fröschel ('08), Blaauw ('09), Loeb ('11), and Parker and Patten ('12) have suggested for varous biological phenomena.

The phototropic reactions of the hamlet, then, are dynamic in character and the stimulus is probably produced by a photochemical process.

C. Exhaustion of the photoreceptors

1. General evidence. A study of the responses both of normal and of blinded fishes to successive stimulations by the same intensity of light often shows a progressive increase in the time of reaction from the first to the last response. A state of complete exhaustion is, of course, reached when no response can be induced. There seems to be, however, no definite ratio between the number of stimulations and the increase in reaction time. Indeed, in two or three individuals the first response was slower than the second, or all responses occurred after about the same interval of time. Even in the latter cases, however, a condition of complete exhaustion was finally effected after a sufficient number of exposures of adequate intensity, but its appearance was abrupt rather than gradual.

2. Photodynamic nature of exhaustion. The process of exhaustion, moreover, appears in general to be photodynamic in character, for the period of resistance of the receptors is lessened by an increase in the intensity of the light and *vice versa.* This inverse ratio is, apparently, similar to that which has just been described in connection with the negative responses.

In experiments on this point it was found necessary in order to produce a state of exhaustion to stimulate the fish for 275 seconds with the light from a 100-watt bulb; but when a 60-watt bulb was substituted, this period was more than doubled, being 562 seconds. Thus a decrease of 40 per cent in the intensity of illumination (from 100 to 60 watts) increased the time of resistance of the photoreceptors more than

100 per cent (from 275 to 562 seconds). Stated in terms of the Bunsen-Roscoe law (product of intensity into time of exposure) the agreement is not very close (27,500 and 33,720), but still it suggests that exhaustion, like stimulation, may be a photo-chemical process.[9]

D. Location of the photoreceptors

Payne ('07, pp. 320–321) in endeavoring to explain the fact that, in Amblyopsis, the negative phototropism is greater when the fish is illuminated from above than when from the side, has suggested that brain and spinal cord may be "affected directly on account of the transparency of the tissues above them." This is equivalent to suggesting that the photoreceptors may be located in the brain and spinal cord; but Parker ('05) has contended that in Ammocoetes the receptors are contained in the integument, for he was able to stimulate this fish by directing light against its belly, which, he argued, contained viscera impenetrable to the light which he employed. He had previously ('03, p. 33) shown that the brain and spinal cord of frogs are also insensitive to light.

In view of this difference of opinion an attempt was made to determine the location of the sensory elements in blinded hamlet. For this purpose the capacity of light (from a 100-watt bulb) for stimulating the integument after it had passed through a considerable mass of muscular tissues was tested. The apparatus used for this experiment consisted merely of a piece of the lateral body muscles, about half an inch in thickness, from another hamlet, held in front of the aperture

[9] An incidental question—the relative fatiguing effects of continuous and of very slowly intermittent light—was also considered. The data of five experiments, of 16–20 exposures each, indicate that continuous illumination and successive illuminations of the same intensity which are separated from each other by one-half or one minute intervals, are about equal in their power to effect complete exhaustion of the cutaneous photoreceptors. The experiments, however, are too fragmentary and the results too inconstant to warrant any general conclusions on this point. If after many tests this relationship should prove to be constant, the difference between it (although there is no ground for assuming a parallelism) and the results of Parker and Patten ('12) is perhaps explained by the fact that a minute, or even one-half minute, interval between exposures is sufficient for the partial recovery of the sense organs. I was ignorant, at the time of this experimentation, of the work of Parker and Patten; otherwise I might have undertaken, as they did, experiments with light which was interrupted at intervals of high frequency. It would be of value to compare from this point of view the photoreceptors of the eye and skin.

of the reflector-box. This tissue constituted the so-called "meat screen." With this apparatus were repeated experiments similar to those on negative phototropism and regional sensitivity which I have described above. The sensitivity of the spinal cord itself to nearly direct stimulation—i.e., without the meat screen—was also tested. The results obtained indicate that the spinal cord of Epinephelus striatus contains no photoreceptive elements, even though light can reach it through the integument and muscles.

It was always possible, contrary to expectation, to cause stimulation with light which had traversed the screen of meat. The reaction varied from the normal response only in its comparative slowness. In one experiment, upon the posterior end of the body, the reaction-time was 28.5 seconds longer than when no "meat screen" was used. This merely shows that light is absorbed as it passes through the tissue, but not sufficiently to render it ineffective. By local stimulation of different areas of the integument with this apparatus, it was even possible to obtain reactions which were consistent with the previously described differences in regional sensitivity to unscreened light. The thickness of the "screen" of tissue (about one-half inch) was the same as that through which light would have to pass in order to reach the spinal cord of an average sized fish. The possibility, then, of such stimulation, provided that the cord contains the appropriate photoreceptors, is assured. Consequently, the presence of half an inch of intervening muscular body-wall cannot be used as a conclusive argument to prove the absence of photoreception in the cord.

The muscles of one side of the body were next removed from a fairly large region anterior to the caudal fin, leaving the cord partially exposed. Uninterrupted light was directed upon the column and cord. A response occurred but it was not quickened, as one would expect if the photoreceptors were located within the cord; on the contrary it was slower by 28.5 seconds than the response of normal fishes. It is significant that this retardation is just the same as that which the "meat screen" caused in experiments where the lateral body wall was not removed. It indicates that in the present experiment the light passed through the cord without affecting it; but that, after traversing the body muscles of the intact side, it stimulated the integument of that side from within. This suggests that the reactions of the blinded fishes were due to photic stimulation of the integument only.

In order to further check this result, the muscles and integument of both sides of the body were removed and the cord and column were

directly illuminated. It was impossible to obtain any reaction. This check experiment was kindly done for me by Dr. Crozier after I left Bermuda.

It appears, then, that the posterior portion of the spinal cord of Epinephelus striatus is not sensitive to electric lights of moderate intensities.

III. CONCLUSIONS

1. At least one strictly marine fish, Epinephelus striatus, possesses an integument which is negatively photosensitive.

2. This sensitivity, expressed by negative reactions, is photodynamic in character, the time of the response being inversely proportional to the intensity of the illumination.

3. The sensitivity is not the same in different regions of the integument; being greatest in the head, less in the tail, and least in the mid-body region.

4. The photoreceptors are completely exhausted by a sufficiently prolonged exposure. The time of illumination necessary to produce exhaustion is in most cases inversely proportional to the intensity of the illumination—a photodynamic relation.

5. The results indicate, also, that the effect of light may be photochemical in nature.

IV. DISCUSSION

The cutaneous sensitivity of fresh water fishes to light has been used by Parker ('05, p. 418) to support Balfour's theory ('81) that the nervous elements of the eye originated from the integument and were once a functional part of it. Provided that the orientation of such cells is constant in the ectoderm and cord during embryonic and racial development, this theory explains the perplexing inversion of the elements of the vertebrate retina. Parker also states ('05) his belief that these light terminals (spinal nerve endings) are degenerate in modern lower vertebrates and also that they may represent the temperature terminals only of higher vertebrates, having lost the photosensitivity which they once possessed. This view involves, if the specialization of function is complete, the assumption that the integument of the higher vertebrates is insensitive to light—unless some other sense organ is regarded as the receptor—and also that the lower vertebrates possess some type of sensory cell which, as a receptor of radiant energy, is at first more or less universal in function (Parker '03, p. 34),

but becomes so changed in the course of phylogenetic development as to function solely as a temperature terminal.

So far as the hamlet is concerned, it should be noted that the integument of its head is sensitive to a temperature change of about 11°C. (from 21° to 10°), because water at 10°C. causes a very precise negative reaction. Since its integument contains receptors for both photic and thermal stimulation, it presents a case in which, according to the view of a primitive and more or less universal radiant-energy receptor, the two functions may inhere in the same nerve terminal; but it should be emphasized that the other view—original independence of heat and light receptors—is likewise defensible. It is, moreover, of interest in connection with the above theory of a specialization of function, that in some of the vertebrates above fishes the integumentary photosensitivity is not entirely lacking. To what extent it persists is as yet known only imperfectly. Graber ('84), Dubois ('90), Korányi ('95) and Parker ('03) have shown that certain Amphibians possess it. Some reptiles also, according to Carlton ('03) and others have skins which are sensitive to light. It is noticeable, too, that as between fishes and amphibians this photosensitivity is negative in the former, but positive in the latter (Parker '03).

More direct evidence of degeneration is seen in Amblyopsis, where, according to Payne ('07) the cutaneous photosensitivity is more pronounced in the young than in the adult. This fish, however, having functional eyes in the younger stages but lacking them in the adult, has probably been independently specialized for a peculiar mode of life and presents an unusual simultaneous degeneration of both optic and cutaneous photosensitivity, rather than ontogenetic evidence parallel to the assumed phylogenetic degeneration.

Parker ('08), after having studied the direction eyes of Amphioxus, concluded that Boveri ('04) was right in claiming that they arise *in situ*, and since the uniform orientation required by Balfour's theory does not in his opinion exist in Amphioxus, he considers the two forms of sensitivity (cutaneous and optic) to be independent of each other. This reasoning presupposes, of course, that the direction eyes of Amphioxus are the more or less immediate predecessors of the rods and cones. At first sight the promiscuous arrangement of the functional eye-cups in Amphioxus does seem to be an objection of Balfour's theory; but it is conceivable that these eye-cups may be secondarily oriented in different directions because light enters the cord from all directions, but that this interference with the uniform orientation of

the photoreceptors, resulting from the special conditions of translucency in Amphioxus, may have had no permanent effect on the orientation of these receptors in vertebrates generally. A completely satisfactory determination of the phylogenetic significance of these cells seems to involve the question of the existence and nature of similar sensory cells within the neural tubes of other vertebrates.

If, hypothetically, according to the Balfour theory, the integument in phylogenetic development gradually loses its sensitivity to light, the cord should by so much gain this property and, as the eyes develop, the cord should in turn lose it, becoming specialized for the coördination and transmission of impulses rather than for receiving them. Of course, these two evolutionary processes might not have been absolutely continuous, and the conditions in the hamlet and some other vertebrates indicate that they may not have been entirely dependent on each other. It is, however, somewhat against Balfour's theory that the degeneration of integumentary photosensitivity does not more perfectly parallel the evolution of directive eyes in the cord,[10] and also that the characteristic vertebrate eyes are coëxistent with a pronounced cutaneous sensitivity in certain fishes, amphibia and reptiles. The probable difference in the structure of the photoreceptive elements in the skin and in the central nervous organs is also, as Parker ('05, p. 418) has pointed out, another possible objection.

The idea of the *in situ* development of the retinal elements in the neural tube and their non-derivation from primitively functional cutaneous elements received corroboration from Parker ('09) when he tested several marine fishes and found the skins of all of them to be insensitive to light. This he interpreted to mean that the vertebrate eye developed not from integumentary elements, but, as Lankester ('80) and Boveri ('04) had suggested, in a manner similar to the *in situ* origin of this organ in ascidian larvae.

The present paper, in demonstrating the existence of cutaneous photosensitivity in one marine fish, makes this argument less convincing; but the presence of sensitivity here, forming at present an exception among marine fishes, affords only a suggestion of the importance of further study on the origin of the eye of vertebrates and its relation to the photosensitivity of the skin. The inconstancy of integumentary photosensitivity may be taken as an argument for its secondary acquisition and against its being a remnant of a primitive function. A final

[10] Crozier ('17) has recently shown that the integument of Amphioxus contains no normal photoreceptors.

solution of these questions, seemingly, must await further studies—from this point of view—upon the development, structure and function of the photoreceptive elements both in the skin and cord. Among such problems the questions of the development of the direction eyes of Amphioxus, and a search for conditions intermediate between them and the typical retinal elements of the lateral eyes, as well as a phylogenetic study of cutaneous photosensitivity, are clearly demanded.

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ON THE MOVEMENTS OF THE EXCISED URETER OF THE DOG

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INTRODUCTION

In the course of an investigation of the effect of certain drugs upon smooth muscle, the ureter of the dog was employed and it was found that the excised ureter of the dog could be made to contract rhythmically for many hours, when placed in a Locke's solution of satisfactory composition.

Since most of our knowledge concerning the movements of the ureter has been obtained either by direct inspection of the intact organ or as the result of the study of pressure changes in it, further study using the excised ureter was thought desirable. Direct inspection of the organ is unsatisfactory and pressure changes in the ureter cannot be considered as being wholly due to influences from within the ureter. Henderson (1) considers that ureteral pressure has nothing to do with the ureter but that it depends entirely upon the kidney. On the other hand, the movements in the excised organ are produced entirely by the ureteral muscles themselves, either by influences originating within the muscles or in their local nerve supply.

EXPERIMENTAL WORK OF OTHERS

The literature dealing with the excised ureter is very meager, the papers by Stern[1] (2), Lucas (3) and Macht (4), (5), (6), (7), (8) being the only important contributions relating to it. Stern used the guinea pig mainly and found that the excised ureter could be made to beat rhythmically in physiological salt solution (0.8 per cent) if the tempera-

[1] An excellent review of the literature of the ureter previous to 1903 will be found in this thesis by Stern. References to the later investigations appear in the present paper.

ture were raised to 42° to 43°C. Lucas worked with the excised ureter of the ox, cat and dog either in Locke's or Ringer's solution attemperatures ranging from 38° to 40°C. In his experiments with the excised ureter he employed the middle third only. He was only able to produce rhythmic contractions of the excised ureteral segment by raising the intraureteral pressure or by means of drugs such as epinephrin or barium chloride. Macht's experiments dealt principally with the behavior of isolated rings of the ureter of the pig, ox and man, in Locke's solution at 37°C. He was quite successful in obtaining spontaneous rhythmic contractions of the ring preparations, suspended either in Ringer's or Locke's solution; whereas when longitudinal preparations were used, he found that they exhibited few or no rhythmic movements.

SCOPE OF PRESENT INVESTIGATION

So far as is known, no attempts have been made to register simultaneously the movements of the several parts of the excised ureter. In the present investigation the movements of the upper, middle and lower thirds of the dog's ureter were simultaneously recorded, and the effects of certain agents and of certain procedures noted. Large dogs were used mainly, although occasionally medium sized dogs were used with success. Before removal of the ureter the dogs were either narcotized or rendered unconscious by mechanical means. They received ether, chloroform or chloretone as anaesthetics. Several were killed with ether or illuminating gas, while others were bled and the ureter removed after exsanguination. The manner of the preparation of the animal, previous to removal of the ureter, seemed to have little effect upon the production of the spontaneous contractions which appeared when the ureteral preparations were mounted in Locke's solution.

METHOD OF STUDYING THE EXCISED URETER

The procedure which was generally adopted for the study of the behavior of the upper, middle and lower third of the ureter was as follows: Under a light but complete ether anaesthesia the right ureter was exposed and carefully separated from fat and other tissues. After being separated from the kidney and bladder, the ureter was immediately put into about 100 cc. of oxygenated Locke's solution (Locke's 2) which was kept at a temperature of about 38°C. It was next divided into three equal parts and the several parts were mounted in a bath containing 200 or 300 cc. of oxygenated Locke's solution, either as lon-

gitudinal segments, longitudinal strips (in which the circular fibers were cut) or as rings which were tied together in chainlike fashion after the manner of Macht (5). The Locke's solution which received the preparations was kept at a temperature of 38°C. Light straw writing levers of uniform construction were used. The weight added to the levers was similar in a given experiment and varied from 0.2 to 0.7 gram; however, 0.2 or 0.3 gram was more generally employed. When the ureter was prepared in the above described manner, the ureteral preparations as a rule would begin to beat regularly in from one-half to one hour.

The effects of oxygen and temperature were studied principally by the use of single longitudinal segments mounted as described above

TABLE 1

Table showing the composition of the various nutrient solutions used

SUBSTANCE	SOLUTION			
	Tyrode's	Locke's 1	Locke's 2	Locke's 3
	grams	*grams*	*grams*	*grams*
Sodium chloride	8.0	9.0	9.0	9.0
Calcium chloride	0.2	0.2	0.24	0.24
Potassium chloride	0.2	0.2	0.42	0.42
Sodium bicarbonate	1.0	0.1	0.3	0.1
Dextrose	1.0	1.0	1.0	1.0
Sodium acid phosphate (monobasic)	0.05			
Magnesium chloride	0.1			
Distilled water	to 1000 cc.	to 1000 cc.	to 1000 cc.	to 1000 cc.

while the effect of nutrient solutions and changes in hydrogen-ion concentration were studied with either single longitudinal segments or longitudinal strips.

NUTRIENT SOLUTIONS EMPLOYED

Four different nutrient solutions were tried. Three were modifications of Locke's formula, while the other was Tyrode's solution. They will be referred to as Locke's 1, 2, and 3, and Tyrode's solutions.

The composition of the nutrient solutions will be found in table 1. The solutions were always made from freshly glass redistilled water and were never used after they were more than one day old.

Locke's 2 was found to be an excellent nutrient solution for the development of the spontaneous rhythm of the excised ureter, as well as for the preservation of its vitality, and it was used almost exclusively in this work.

CHARACTER OF URETERAL MOVEMENTS

To determine the character of the ureteral movements, their rate and amplitude, longitudinal segments were employed. All parts of the ureter were found to be capable of spontaneous movements when mounted in oxygenated Locke's solution.[2]

Two varieties of contractions occur, namely, small and large contractions. The small contractions as a rule appeared sooner than the large contractions, sometimes as soon as five minutes after mounting, while the appearance of the large contractions varied from thirty minutes to three hours after the ureteral preparations were put in the Locke's solution. After the small contractions appear they do not increase markedly in height, whereas the large contractions are usually relatively small at first and increase in a steplike fashion to a marked size. The contractions may appear in the various segments about the same time after mounting but not necessarily so. They seem to follow no rule. In certain tracings only the large contractions may be found; while in others only the small appear. The large contractions are as a rule quicker in contracting and relaxing than the small contractions; the former consume about one and one-half to four seconds in contracting and almost at once begin to relax, the relaxation requiring about seven to seventeen seconds. Some small contractions simulate the large contractions in the time required to contract and relax; while others are more sluggish, requiring about six to eight seconds to contract and about twenty to thirty seconds to relax. Moreover, they may remain in contraction several seconds before relaxation occurs. Sometimes small contractions are superimposed upon the large ones.

Small contractions predominated in the kidney third, while in the middle and bladder thirds large contractions were most frequently found. It was thought that, since the segment preparations contain intact circular fibers, possibly the small contractions may be caused by them. To test this theory the circular fibers of longitudinal segments were cut through transversely, so that a longitudinal strip preparation was made, and it was found that both small and large contractions still appeared in some cases (fig. 1). Apparently both varieties are produced by the longitudinal muscles.

Ring preparations of the upper, middle and lower parts of the ureter, following the technic of Macht (5), produced small spontaneous rhythmic movements in every part (fig. 2). Too few experiments were

[2] Locke's solution always refers to Locke's 2 unless otherwise stated.

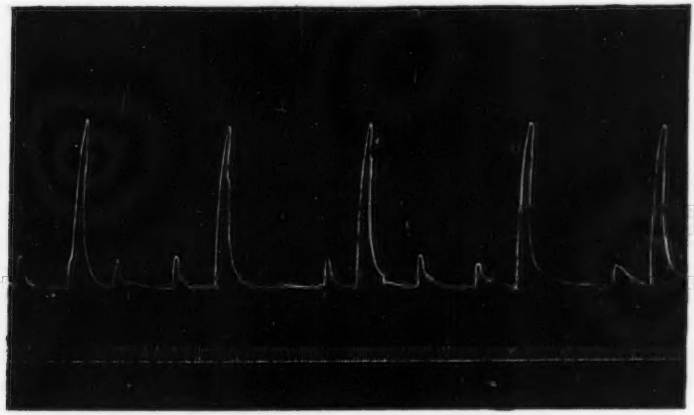

Fig. 1. Spontaneous contractions produced by a longitudinal strip (circular fibers cut) middle third, excised ureter dog, in Locke's 2.

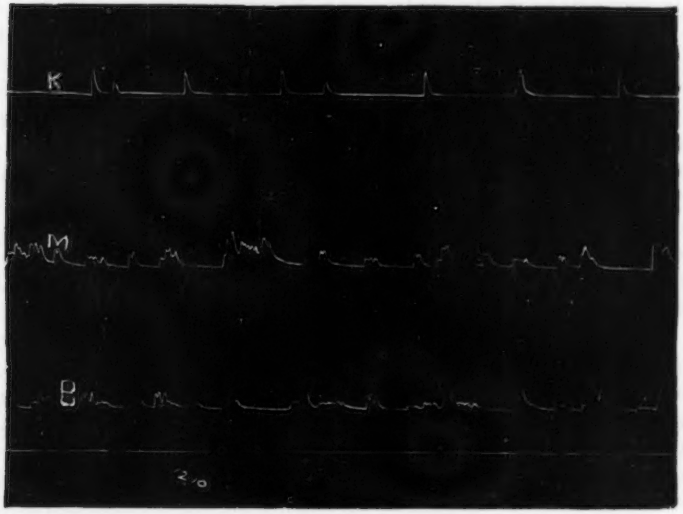

Fig. 2. Spontaneous contractions produced by ring preparations of kidney *K*, middle *M* and bladder *B* third, excised ureter dog, in Locke's 2.

made to enable one to conclude whether the contractions which were obtained in this way could be grouped into distinct varieties.

RATE OF CONTRACTIONS

The rate of the contractions in the various segments varies from four to fifty in ten minutes. In any segment the contractions are relatively infrequent at first and later become more frequent. When the two varieties of contractions are present, one or the other predominates. The increase in the rate of the large spontaneous contractions is generally accompanied by an increase in amplitude. This is true, however, only for the large spontaneous contractions which appear early, and does not hold true for the effect after the application of drugs to the segment. The changing of the Locke's solution, and changes in temperature and in the supply of oxygen, are the principal factors affecting the rate after a uniform rate has become established. The contractions of the various segments are not synchronous.

VARIATIONS IN THE AMPLITUDE OF CONTRACTIONS

The amplitude of the contractions of the several segments varied. As a rule, segments from the middle and bladder third contracted more vigorously than segments from the kidney third. The amplitude of the contractions seemed to depend to a large extent upon the degree of trauma entailed by the necessary handling of the organ in the preparation of the experiment.

In addition to the two types of contractions which have already been described, another type of movement was noticed in four experiments in which the kidney segment, and one in which the middle segment was used, namely, relaxation (fig. 3). These segments were lightly weighted with 0.2 gram weights. Small contractions predominated in these experiments but at certain times relaxation occurred. In two experiments the relaxation was at times independent of the small contractions and occurred at fairly regular intervals, the small contractions occurring at the rate of about one per minute; whereas relaxation occurred about every three to four minutes. In one of the above experiments, after one hour, only small contractions occurred. However, in this experiment relaxation again occurred after epinephrin and barium chloride; the relaxation being especially pronounced after the latter. In the other two experiments relaxation was associated with a contraction, relaxation usually appearing between two contrac-

tions. From these experiments it is seen that, in the upper part of the ureter, relaxation may occur independently of contraction. Perhaps a distinct mechanism is involved in the production of this phenomenon. A possible explanation for the relaxation which occasionally occurs in longitudinal segments is that the elongation is due to the contraction of the circular muscles.

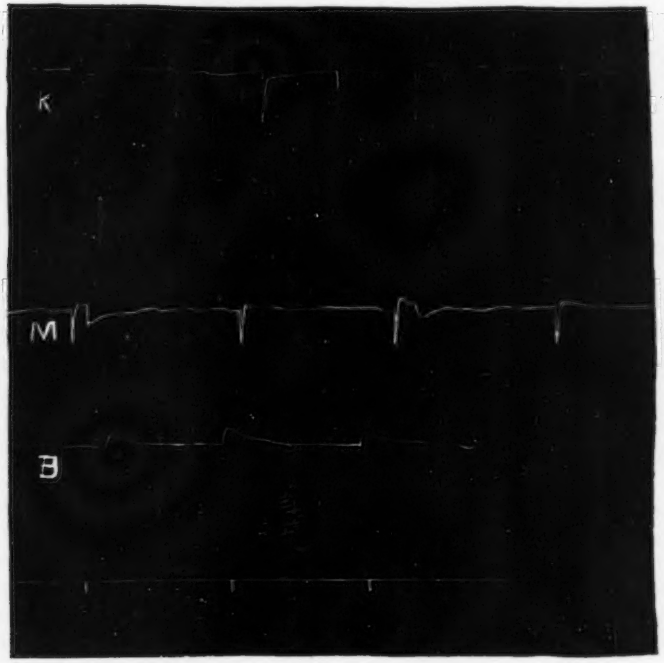

Fig. 3. Movements produced by longitudinal segments of kidney *K*, middle *M* and bladder *B* third, excised ureter dog, in Locke's 2. Note relaxation in kidney and middle segments. Upstroke denotes contraction; down stroke, relaxation.

EFFECTS OF VARYING THE TEMPERATURE

Experiments with longitudinal segments taken from the middle third in which the temperature was varied, either raised or lowered within certain limits, showed that the ureter reacts in an analogous manner to

that of other smooth muscle. Lowering the temperature decreased the rate, tonus and amplitude; while increasing the temperature within certain limits increased the rate, tonus and amplitude. The most favorable range of temperature for the development of spontaneous movements was between 37° and 39°C. One segment ceased beating at 35°C., while another kept on beating slowly for some time at 28°C.

EFFECTS OF VARYING THE OXYGEN SUPPLY

Previous investigators are not in accord as to the influence of oxygen on the excised ureter. Stern (2) and Macht (5) found that oxygen had a favorable influence on the activity of the ureter; while Lucas (3) considered that the ureteral contractions which he obtained seemed to be developed less satisfactorily in oxygenated than in unoxygenated solutions. The influence of oxygen on the ureteral movements was investigated, both on single segments and on the several segments beating together in the same solution. It was found that lack of oxygen prevents or delays the appearance of the spontaneous contractions, and that when the segments are beating rhythmically and the oxygen supply is shut off, the effect was practically the same as that produced by lowering the temperature, namely, a decrease in rate and amplitude.

EFFECTS OF CERTAIN DRUGS

Inasmuch as the spontaneously beating excised ureter lends itself readily to the study of drugs, and since the action of certain drugs reveals the presence or absence of nerves, an effort was made to determine whether nerves exist throughout the ureter or only in part.

Engelmann (9) could find no ganglion cells in the upper part of the rabbit's ureter. He concluded that the cause of the normal periodic movements of the ureter is found in the muscle substance itself.

According to Fagg (10) stimulation of the hypogastrics is followed by a motor effect on the ureter, either quickening the normal rhythm of the contraction by the production of groups instead of single contractions or by the production of groups of contractions in a previously motionless ureter, while stimulation of the pelvic visceral nerves was without effect on the ureters. He concludes therefore that the ureters are apparently solely innervated by the hypogastrics.

Langley and Anderson (11) from dissections on cats, rabbits and dogs, found that the ureter was supplied by a branch from the main hypogastric nerve.

Elliott (12) could not obtain constriction of the ureter of the dog by stimulation of the hypogastric and states that although the ureters are supplied by branches from the sympathetics, the connection remains little more than a potentiality.

Macht (5) (6) advances pharmacological proof that the ureter is innervated through the autonomic nervous system.

For the purpose of ascertaining the nerve distribution, epinephrin, barium and apocodeine were employed. Epinephrin, in as high a dilution as 1 to 1,000,000, caused in every segment an immediate marked increase in the tonus, together with a slight increase in the rate and amplitude. After the amplitude increased markedly, the rate decreased. If small contractions were present, they usually disappeared

Fig. 4. Effect of epinephrin on longitudinal segments of kidney *K*, middle *M* and bladder *B* third, excised ureter dog, in Locke's 2.

and the number of large contractions increased. No difference in the effect on the kidney, middle and bladder third could be noted (fig. 4). Barium chloride, in dilutions of 1 to 10,000 to 1 to 5,000, caused practically the same effects on the several segments as epinephrin, namely, an increase in tonus, rate and amplitude. The latent period or the time elapsing between the time of administration of the barium and the response to it, is longer than for epinephrin. This, however, is largely a question of dosage (fig. 5). Apocodeine first stimulates then depresses ureteral activity. After apocodeine, in concentrations of 1 to 10,000 to 1 to 5000, the epinephrin effects are abolished or very much lessened; while the response to barium is practically normal (fig. 6). These effects hold true for every part of the ureter.

From the above findings we must conclude that since epinephrin in dilute solution stimulates all parts of the ureter to greater activity, sympathetic or autonomic nerve endings are present throughout the

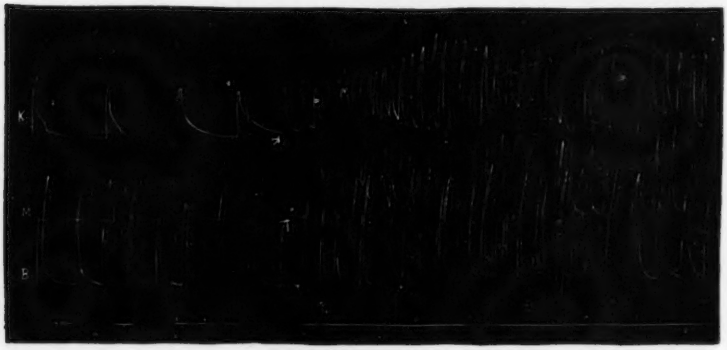

Fig. 5. Effect of barium chloride on longitudinal strips of kidney *K*, middle *M* and bladder *B* third, excised ureter dog, in Locke's 2.

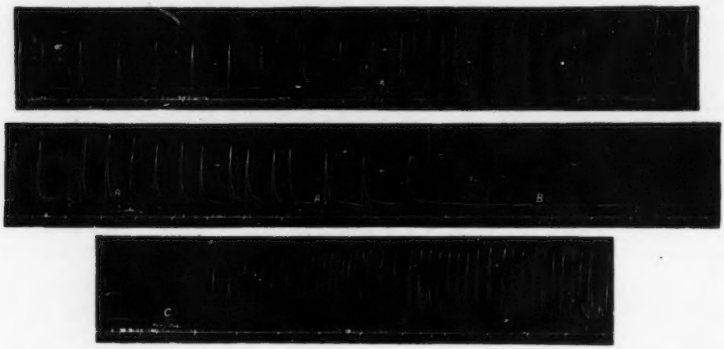

Fig. 6. Effect of epinephrin and barium chloride after apocodeine on longitudinal strip of kidney third, excised ureter dog, in Locke's 2. At each of the points marked *A*, 20 mgm. of apocodeine added; at *B* 1 cc. of 1 to 2000 epinephrin; at *C* 20 mgm. of barium chloride.

entire ureter, as epinephrin has been shown by others to act upon these structures. The absence of an epinephrin effect after apocodeine indicates a paralysis of structures stimulated by epinephrin, namely, the autonomic nerve endings.

In addition to the above, nicotine, pilocarpine, atropine, cocaine and novocaine were tried on single, longitudinal strip or segment preparations in a small number of experiments. The results with nicotine, pilocarpine and atropine were unsatisfactory; either no effects were noted or at best only a questionable stimulating effect from nicotine and pilocarpine. Cocaine in 1 to 20,000 to 1 to 10,000 dilution and novocaine in more concentrated solution, on single segment preparations, were shown to stimulate ureteral activity; the effect of small doses was practically the same as that produced by epinephrin.

EFFECT OF VARYING THE HYDROGEN-ION CONCENTRATION OF THE NUTRIENT SOLUTION

The effect of varying the hydrogen-ion concentration[3] was studied by modifying Locke's 2 with carbon dioxide, sodium acid phosphate (monobasic), and one-tenth normal hydrochloric acid.

Macht (5) (8) working mainly with ring preparations of the ox ureter found that a urine-Locke mixture in the proportion of about 1 to 10 was admirably suited for the development of the spontaneous contractions. He used fresh acid urine and considers that the urine-Locke mixture was superior to the simple Locke's solution by reason of the greater hydrogen-ion concentration of the urine-Locke mixture. He does not state the composition of the Locke's solution which he used. It appeared to him that a slightly acid medium was necessary for the maintenance of the normal ureteral contractions. In support of this he cites an experiment in which the pig's ureter, which was beating poorly in the Locke's solution, was improved by increasing the hydrogen-ion concentration to 6.8 by the addition of urine. When sodium bicarbonate was added until the hydrogen-ion concentration was reduced to 7.8 the contractions ceased.

The Locke's solution (Locke's 2) which was used for the most part in the present investigation had a hydrogen-ion concentration of 8 or 8.1.[4] Since this solution was found to be best suited for the production and maintenance of the spontaneous contractions of the excised ureter of the dog, it appeared that like other tissues of the body, an alkaline

[3] The reaction of a fluid may be expressed as pH, a neutral solution having a pH value of 7. As the hydrogen-ion concentration increases the pH value decreases and vice versa.

[4] Determined colorimetrically. I am indebted to Mr. J. W. McBurney of the Division of Chemistry for many of the hydrogen-ion determinations of the nutrient solutions used in this work.

medium was best suited for this purpose. The Locke's 2 used in the earlier experiments was made up from stock solutions and in these experiments the hydrogen-ion concentration was found to be 8.3 while in the later experiments stock solutions were not employed and the hydrogen-ion concentration was 8.0 or 8.1; Locke's 1 had a pH value of 7.9;[5] Locke's 3 was 7.5 and Tyrode's was either 8 or 8.1. All of these solutions therefore were distinctly alkaline.

Although it was found that Locke's 2 and Tyrode's solution had the same hydrogen-ion concentration, Tyrode's was not a favorable medium for the excised ureter. Tyrode's solution differs from Locke's solution mainly in containing sodium acid phosphate and magnesium chloride. Both of these substances were found to be depressant to the ureter when added to Locke's 2.

In one experiment 10 mgm. of magnesium chloride added to 200 cc. of Locke's 2 stopped the ureteral movements in several minutes. This amount is only one-half that found in Tyrode's. Similarly, sodium acid phosphate in one-hundredth of one per cent concentration in Locke's 2 will sometimes stop the beating ureter. However, this is double the concentration that appears in Tyrode's.

Locke's 1 and 3 differed from Locke's 2 mainly in having a greater hydrogen-ion concentration, however, the difference in reaction between Locke's 1 and Locke's 2 was not marked.

By the use of strip preparations from the middle third of the ureter it was found in certain experiments that the contractions which were produced by immersion of the strip in Locke's 2 could be promptly stopped by increasing the hydrogen-ion concentration to 6.5 by means of carbon dioxide. Changing the strip to Locke's 2 (pH 8.1) restored the contractions at once (fig. 7). Strips which refused to beat in Locke's 2 could not be made to beat by adding carbon dioxide until the solution became slightly acid.

[5] This pH value of Locke's 1 is much higher than that reported by Rona and Neukirch (Arch. f. d. gesammt. Physiol., 1912, cxlviii, 273). The value which they obtained may be due to the use of old distilled water which had absorbed considerable carbon dioxide. It may also be due to the fact that the observation may have been made after the Locke's 1 had been kept for some days. This is probable since their findings and the above agree fairly well for the Tyrode's but differ markedly for Locke's 1. The buffer effect of Tyrode's was found to be considerable so that its pH value changes little after several days while Locke's 1 buffer effect was found to be slight and as a consequence its pH value markedly decreases after several days.

By increasing the hydrogen-ion concentration to 6.2 with one-tenth normal hydrochloric acid the spontaneous contractions would soon cease. They could be reëstablished by using an amount of one-tenth normal sodium hydrate sufficient to neutralize the added acid (fig. 8).

Sodium acid phosphate added to Locke's 2 in amounts sufficient to increase the hydrogen-ion concentration to 7 or 6.6 caused the beating

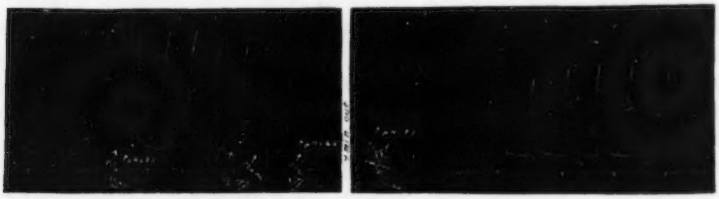

Fig. 7. Effect of carbon dioxide on longitudinal strip from middle third excised ureter dog, in Locke's 2.

Fig. 8. Effect of hydrochloric acid followed by sodium hydroxide on longitudinal strip from middle third excised ureter dog, in Locke's 2.

Fig. 9. Effect of sodium acid phosphate followed by sodium bicarbonate on longitudinal strip from middle third, excised ureter dog, in Locke's 2.

ureteral strip to become quiescent after a brief period of stimulation. The normal rhythm could be restored by the addition of sodium bicarbonate (fig. 9).

In contrast to the above it was found in other experiments that a preparation which was beating well in Locke's 2 continued to beat regularly when one-tenth normal hydrochloric acid was added in an

amount sufficient to increase the hydrogen-ion concentration to 7.2, or by increasing the hydrogen-ion concentration to 6.8 or 6.6 by means of sodium acid phosphate, the ureteral contractions continued, although they became fewer and somewhat irregular.

A strip preparation which refused to beat in Locke's 2 was transferred to a urine-Locke mixture (one part urine to ten of Locke's 2) and immediately the preparation began to beat. The rhythm, however, was irregular. The hydrogen-ion concentration of this mixture was 6.9. After beating for some time in this mixture, sodium bicarbonate was added until the hydrogen-ion concentration was decreased to 7.4. The contractions then became more regular. Further decreasing the hydrogen-ion concentration to 8 had the effect of increasing the amplitude of the contractions and slightly decreasing their rate. The contractions continued even though the hydrogen-ion concentration was decreased to 8.4.

From these experiments it is seen that although an alkaline medium is best adapted for the production and maintenance of the rhythmic contractions of the excised ureter of the dog, the ureter will sometimes beat in a slightly acid medium and that in the urine-Locke mixture its acidity should not be considered as the probable cause of the rhythmic movements which are produced by it.

SUMMARY

Longitudinal segments and strips, as well as ring preparations of the kidney, middle and bladder third of the excised ureter of the dog were found to beat rhythmically in oxygenated Locke's solution which had a hydrogen-ion concentration of 8 or 8.1.

Two distinct varieties of contractions appeared, namely, a large and a small variety. These contractions were produced either by longitudinal segments, or by longitudinal strips. In the latter the circular fibers are cut, so that both types are apparently produced by the longitudinal muscles. Relaxation which was independent of contraction was seen in a few experiments in which longitudinal segments were used.

The rate and amplitude of the contractions varied but as a rule longitudinal segments from the middle and bladder third contracted more vigorously than those from the kidney third.

Epinephrin and barium produced an increase in the tonus, rate and amplitude of the beating segments. Apocodeine first stimulated and

then depressed ureteral activity. After apocodeine the epinephrin effect is abolished or lessened while the response to barium is practically normal, thus indicating the presence of autonomic nerve endings throughout the ureter.

In a limited number of experiments either no effects were noted with nicotine, pilocarpine and atropine, or only a questionable stimulating effect from nicotine and pilocarpine. Cocaine and novocaine both stimulated ureteral activity.

As a rule increasing the hydrogen-ion concentration slightly by means of carbon dioxide, hydrochloric acid or monobasic sodium acid phosphate stopped ureteral movement. The contractions thus stopped could be reëstablished by restoring the original hydrogen-ion concentration by changing to an alkaline Locke's or by means of sodium hydroxide or sodium bicarbonate.

While the ureter will occasionally beat in a slightly acid medium for some time, an alkaline medium is best adapted for the production and maintenance of the spontaneous contractions of the excised ureter of the dog.

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THE EFFECT OF ETHER ANAESTHESIA, THE EMOTIONS AND STIMULATION OF THE SPLANCHNICS ON THE CATALASE CONTENT OF THE BLOOD

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As a result of the work of Paul Bert (1), Arloing (2), and particularly of Alexander and Cserna (3), it is now generally accepted that the initial effect of anaesthetics on the respiratory exchange is to cause an increase in oxygen consumption and carbon dioxide production; however, after the preliminary stimulating action has passed the effect is to produce a decrease in the consumption of oxygen and the production of carbon dioxide, so on the whole it may be said that anaesthetics produce a decrease in the respiratory exchange. These observations indicate that during the excitement stage of ether anaesthesia oxidation in the body is increased, while during the rest of the stages it is decreased. The fall of body temperature during anaesthesia, the occurrence of acetone in the breath and urine (4), the appearance of glycosuria (5), the increase in unoxidized sulphur (6), are taken as further evidence that the oxidative processes are decreased or rendered defective by anaesthetics.

It has been shown in this laboratory that when oxidation is increased or decreased in an animal by increasing or decreasing the amount of exercise, by thyroid feeding or by the fighting emotions, there is a corresponding increase or decrease in the catalase of the muscle, hence the conclusion was drawn that there exists a very close relationship between catalase content and amount of oxidation. Evidence was also presented to show that catalase formed in the liver and given off to the blood during exercise, in combat, etc., is carried to the muscles and used in the increased oxidation, presumably making it possible, and that the output from the liver is controlled by stimuli received over the splanchnics (7).

The present investigation was begun in an attempt to obtain further evidence regarding the production and function of catalase. If cata-

lase is concerned in oxidation then the amount in an animal should be increased during the excitement stage of ether anaesthesia and decreased during the other stages, since oxidation is increased during the excitement stage and decreased during the other stages. Dogs and cats were the animals used in this investigation. The amount of catalase in the blood was determined according to the following method. One-half cubic centimeter of blood was added to hydrogen peroxide in a bottle and the oxygen gas liberated in ten minutes was conducted through a rubber tube to an inverted vessel previously filled with water. After reducing this volume of gas to standard atmospheric pressure the resulting volume was taken as a measure of the catalase content of the 0.5 cc. of blood. In determining the catalase content of the blood of dogs 45 cc. of hydrogen peroxide were used and in determining that

TABLE 1

Under the different stages are given the number of cubic centimeters of oxygen liberated in ten minutes from hydrogen peroxide by 0.5 cc. of blood of dogs and cats in the respective stages of anaesthesia

ANIMALS	ADMINISTRATION OF ETHER					RECOVERY FROM ETHER				
	First stage	Second stage	Third stage	Fourth stage	Percentage decrease	Fourth stage	Third stage	Second stage	First stage	Percentage increase
Dog 1.....	70	65	55	48	32	48	60	68	75	56
Dog 2.....	62	60	53	42	29	42	45	60	59	40
Cat 1.....	875	775	725	600	31					

of cats 500 cc. This larger amount of peroxide was used in the case of cats because of the high catalase content of their blood. The hydrogen peroxide used in all these determinations was prepared by diluting commercial hydrogen peroxide with an equal volume of distilled water. A full description of the method may be found in a previous publication.

The effect on catalase of increasing and of decreasing the depth of anaesthesia. The data in table 1 were obtained from dogs that had been injected with morphia and slightly etherized previous to the introduction of the cannula into the trachea. The tracheal cannula was connected by means of a rubber tube with an ether bottle and the apparatus so arranged that the amount of air and ether administered could be changed at will. Immediately after introducing the cannula into the trachea about 3 cc. of blood were taken by means of a hypodermic syringe from the external jugular and the amount of catalase in 0.5 cc. of this blood was determined according to the method given. Sim-

ilarly determinations were made after 15, 30 and 45 minutes respectively. These data are given in table 1 under "administration of ether." The dogs were regarded as being in the first stage of ether anaesthesia immediately after the introduction of the tracheal cannula, and in the second, third and fourth stages after 15, 30 and 45 minutes respectively. These different stages were chosen more or less arbitrarily and used as a convenient method for the presentation of the results. During the fourth stage the dogs were very deeply under the ether and had almost ceased to breathe. After taking the blood sample for this stage the apparatus was so adjusted that air containing but little ether was administered and the animal was permitted slowly to recover from anaesthesia. During this period of recovery determinations were also made of the catalase content of the blood at intervals of about 15 minutes. The results of these determinations are given in the table under "recovery from ether." The catalase of the blood of a cat was also determined during the period of "administration of ether."

It will be seen that the catalase content of the blood decreased during the periods of administration of ether, as is indicated by a decrease in the amount of oxygen liberated, and increased during the periods of recovery from ether, and that the catalase was decreased by about 30 per cent in the fourth stage during the administration of ether and had returned almost to the normal amount in the first stage of anaesthesia during the period of recovery from ether. The question that naturally suggests itself in this connection is, does the ether during the period of administration destroy the catalase of the blood or simply inhibit its action so that during the period of recovery when the ether is removed from the blood the catalase returns to its normal strength? The following experiments were carried out in an attempt to answer this question.

The effect on catalase of exposing blood in vitro to ether vapor under pressure. Two dogs were quickly etherized and bled to death. The defibrinated blood from these animals was exposed to ether vapor under a pressure of 10 mm. of mercury at 36°C. for 60, 120, 180 and 240 minutes respectively. The catalase content of the unexposed blood and of the blood exposed for the different periods was determined. Results of these determinations are given in table 2 under "dog blood" after specimens 1 and 2. It will be seen that the average amount of oxygen liberated by 0.5 cc. of the normal or unexposed blood was 63 cc., that liberated after exposure of blood to ether vapor for 60, 120, 180 and 240 minutes respectively was 53, 43, 42 and 39 cc. of oxygen,

hence the catalase content of the blood was decreased about by 38 per cent as is indicated by a decrease from 63 to 39 cc. of oxygen. The blood of a cat was similarly exposed and the results are given in the table under "cat blood." It will be seen that the exposure of cat blood to ether vapor decreased the catalase content by about 34 per cent. In performing the preceding experiment great care must be taken to see that none of the liquid ether comes in contact with the blood for this will destroy the catalase very quickly. Other experiments were carried out in which the catalase of the blood was lowered by administering ether to the animal. When the catalase was decreased by about 40 per cent which was determined from a sample of blood taken from

TABLE 2

Under "normal" and "exposed to ether" are given the number of cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of dog's blood and cat's blood exposed to ether vapor for the respective intervals of time. Under "bubbling of air" are given the amounts of oxygen liberated by the blood previously exposed to ether that had had air bubbled through it for the respective periods

	NORMAL	EXPOSED TO ETHER				PERCENTAGE DECREASE	BUBBLING OF AIR			
		60′	120′	180′	240′		60′	120′	180′	240′
Dog blood:										
Specimen 1	65	55	44	44	40	40	42	40	38	40
Specimen 2	60	51	42	40	38	36	38	40	37	36
Average	63	53	43	42	39	38	40	40	37	38
Cat blood:										
Specimen 1	875	775	725	600	575	34	575	525	565	500

the jugular, the animal was bled to death. After defibrination one sample of the blood was exposed to a vacuum to remove the absorbed ether; oxygen gas was bubbled through another and air through another. The catalase of the blood was not increased by any of these treatments, as is the case when animals are permitted to recover from ether by breathing air. The conclusion is drawn that the decrease in the amount of catalase in the blood of animals during the period of administration of ether is due to a destruction of the catalase and not to an inhibition of its activity. The following experiments were carried out in an attempt to find an explanation for the increased catalase content of the blood during the period of recovery from ether.

The effect of stimulating the splanchnics on the output of catalase from the liver. When the dog was well under ether, or in what is referred

to as the third stage of anaesthesia in table 3, the abdominal wall was opened and a sample of blood was taken from the hepatic vein and the catalase content of 0.5 cc. of this determined. The two splanchnic nerves were then dissected out where they emerge from under the diaphragm and shield electrodes placed on them. Very weak stimuli from an induction coil were applied for about five seconds at intervals of about fifteen seconds. This intermittent stimulation was continued for about fifteen minutes. At the end of this time another sample of blood was taken from the hepatic vein and the catalase content of 0.5 cc. determined. Similarly determinations were made at the end of 30, 45 and 60 minutes of stimulation. The results of the determinations are given in table 3 under "third stage of anaesthesia" and 15, 30, 45

TABLE 3

Under "third stage of anaesthesia" and periods of "stimulation of splanchnics" are given the number of cubic centimeters of oxygen liberated from 50 cc. of hydrogen peroxide in ten minutes by 0.5 cc. of blood from dogs in the third stage of anaesthesia and with their splanchnics stimulated for the respective periods

DOG	THIRD STAGE OF ANAESTHESIA	STIMULATION OF SPLANCHNICS				PERCENTAGE INCREASE
		15′	30′	45′	60′	
Dog 1	48	57	54	60	61	27
Dog 2	50	68	70	75	70	40
Dog 3	50	60	65	70	75	50
Average	49	62	63	68	69	39

and 60 minutes of stimulation of splanchnics. It will be seen that the average amount of oxygen liberated by 0.5 cc. of blood from dogs in the third stage of anaesthesia was 49 cc., that liberated after stimulating the splanchnics for 15, 30, 45 and 60 minutes was 62, 63, 68 and 69 cc. respectively. This is taken to mean that the stimulation of the splanchnics increased the output of catalase from the liver by about 40 per cent. Precaution of course was taken to see that the animal was kept in about the same state of anaesthesia throughout the experiment. The following experiment was carried out to determine if it was possible to increase the output of catalase from the liver by prolonging the period of excitement in ether anaesthesia.

The effect on catalase of prolonging the excitement stage in ether anaesthesia. Ether was administered to a dog by placing over the nose of the animal a cone shaped vessel attached to an ether bottle by a rubber

tube. A large amount of air was mixed with the ether so that the dog struggled during its administration. After permitting the animal to struggle about twenty minutes during the administration of this mixture the supply of air was decreased and the amount of ether was increased. As soon as the dog was etherized a sample of blood was quickly taken from the hepatic vein and the catalase content of 0.5 cc. of the sample determined. Similarly determinations were made of the catalase of the blood of two other dogs which had struggled violently during the excitement stage of the administration of ether and of two dogs which had been injected with morphia previous to the administration of ether. In the morphinized animals there was very little or no excitement stage during the administration of ether. The results of the determinations are given in table 4. It will be seen that the average amount

TABLE 4

Under "normal" and "prolonged excitement" are given the number of cubic centimeters of oxygen liberated in ten minutes from 50 cc. of hydrogen peroxide by 0.5 cc. of blood of normal dogs and of dogs which had undergone an excitement stage of about twenty minutes at the beginning of the administration of ether

DOG	NORMAL	PROLONGED EXCITEMENT	PERCENTAGE INCREASE
Dog 1	68	152	123
Dog 2	72	199	210
Dog 3	60	136	126
Average	66	162	153

of oxygen liberated by 0.5 cc. of blood from the dogs in which there was very little or no excitement stage of anaesthesia was 66 cc., while the average amount liberated by the dogs in which the excitement stage of anaesthesia had been prolonged was 162 cc. These results are interpreted to mean that the struggle during the administration of ether had increased the output of catalase into the blood in a manner similar to what occurs in combat. We have determined in connection with another problem the catalase content of the blood of at least forty dogs that had been morphinized previous to etherization and 0.5 cc. of the blood of none of these animals liberated more than 70 cc. of oxygen from 50 cc. of hydrogen peroxide, so that we are satisfied that the catalase content of the blood of dogs that do not undergo the prolonged and violent excitement stage of anaesthesia is low.

It is assumed that the increase in the catalase of the blood during

recovery from ether is brought about in the same manner that it is by prolonging the excitement stage of ether anaesthesia or by stimulating the splanchnics, namely, by increasing the output of catalase from the liver into the blood.

The fact that unconsciousness follows very quickly after cutting off the blood supply to the brain and that the conductivity of a nerve is suspended when deprived of oxygen indicates that oxygen is very essential in the normal functioning of nervous tissue, as in other tissue. Oxygen, per se, of course is not the essential thing but oxidation. When the brain or a nerve is deprived of oxygen there is a decrease in oxidation and an increase in incompletely oxidized substances resulting in an interference with normal functioning. The fact that ether destroys the catalase of the blood and presumably of the nervous tissue, thus producing a decrease in oxidation, suggests that this effect may be the means by which ether produces anaesthesia. It is recognized that chloroform is a more powerful and a more dangerous anaesthetic than ether. In some unpublished results we observed in chloroform poisoning that the catalase of the liver as well as of the blood was decreased, as is the case in phosphorus poisoning. The more powerful effect of chloroform as an anaesthetic may be due to its harmful effect on the liver whereby the output of catalase from this organ is decreased in addition to the destructive effect on the catalase of the blood. According to the above hypothesis the decrease in oxidation in the nerve centers is produced by the destruction of catalase by the anaesthetic thus bringing about the depressed condition of the nervous system or anaesthesia. On cessation of the administration of the anaesthetic the amount of catalase in the center is increased owing to absorption of catalase from the blood and hence oxidation is increased. With increased oxidation in the centers irritability returns, the splanchnics again take up their function in producing an increased output of catalase from the liver into the blood. The experimental facts we have at the present time bearing out this hypothesis are enumerated in the conclusions.

CONCLUSIONS

1. The catalase content of the blood decreases during the administration of ether and increases during the recovery from ether. The decrease is due to the destruction of catalase by ether, the increase to an increased output of catalase from the liver. The decrease in catalase during the administration of ether may be the cause of the decreased

oxidation during anaesthesia, while the increased output of catalase from the liver during recovery may account for the increased oxidation during this period.

2. Blood catalase is destroyed in vitro by exposure to ether vapor as happens in vivo during the administration of ether; however, in the former case catalase is not restored to the normal amount when the ether is removed by bubbling air or oxygen through the blood as occurs in vivo when the animal is permitted to recover from the effect of ether by breathing air.

3. The catalase content of the blood can be increased by prolonging the excitement stage of ether anaesthesia or by stimulating electrically the splanchnic nerves distributed to the liver. The increase in catalase may account for the increased oxidation during the excitement stage of ether anaesthesia just as the decrease in catalase may account for the decreased oxidation during the rest of the stages.

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FACTORS INFLUENCING THE INTERCHANGE OF FLUID BETWEEN BLOOD AND TISSUE SPACES

I. Blood Pressure

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GENERAL INTRODUCTION

This work is the outcome of an interest in the effects of muscular work on the body as well as in the problems of oedema. It is a continuation of work done on the absorption of tissue fluid (1), (2). Some investigators who have examined the effects of exercise on the blood have reached conclusions to which the present author could not subscribe without further investigation of the effects of some factors involved. In muscular work two factors are involved which might alter the fluid content of the blood, viz., altered blood pressure and activity of the muscles. The effects of both of these factors have been investigated.

In investigating the problems of interchange of fluid between the blood and tissue spaces, one might determine the changes in the lymph flow from the part, the water content of the tissues or changes in the blood. In dealing with the body as a whole the investigation of the lymph flow from the thoracic duct is not entirely satisfactory since alterations in the flow may not be synchronous with the exciting cause and many observers have found changes in the blood in a very short time. Also an increased flow of lymph does not necessarily mean an increased concentration of blood, e.g., in hydraemic plethora. On the other hand, if the blood be examined, one is met with the difficulty that the methods are so subject to error that unless large changes are found one cannot put much weight in the results. In the present work the changes in the blood have been chiefly used, although the other methods have been used in part. Before describing the methods used we must first discuss the homogenicity of the blood. Some authors, Hawk (3), Schneider and Havens (4), have explained their results on the basis that there are masses of corpsucles stored away in certain

organs that may be liberated in time of need while Lamson (5), (6) believes the number of corpsucles is altered by a splitting of old corpuscles or a production of new ones as well as by storage in the liver. We believe that the work of von Lesser (7) and of Cohnstein and Zuntz (8) shows that the composition of the blood in all large arteries and veins at any given time is practically the same. If the capillaries of some organs contain large numbers of corpsucles which might be liberated at any time and pass into the general circulation, it is evident that the investigation of the number of corpuscles or the hemoglobin content of the blood is useless in trying to determine the fluid interchange between blood and tissues. In looking for such masses of corpuscles it is possible to compare the capillary blood of various organs with that in the larger vessels. A number of experiments of this kind has been made but so far the capillary blood has always been practically identical in hemoglobin content with the carotid blood. The details of this work will be published later. There is, however, another way in which this problem may be approached. If there are such masses of corpuscles hidden in some organ, one would believe they would be called out into the general circulation in time of stress such as would occur after hemorrhage, or could be washed out into the general circulation by making the animal plethoric by means of Ringer solution. The following figures (table 1) are compiled from the data given in the paper referred to above (2) in which the effects of hemorrhage and of plethora on the blood corpuscles were investigated. The hemoglobin content and corpuscle volume were determined from samples of carotid blood. The total volume of the blood was determined by subsequently washing out the blood vessels and determining the dilution from the hematin values.

A comparison of the hematokrit and hemoglobin values shows that the hemoglobin varies with the corpuscle mass and the table shows that if there are masses of corpuscles hidden in any organ they do not come into the general circulation when most needed (after hemorrhage) nor can they be washed into the general circulation by making the animal plethoric. The columns showing the total mass of corpuscles left and found are of less value since the total volume of the blood was calculated from the hemoglobin value. They do, however, serve as a check on the accuracy of the work. Some who have studied alterations in the blood using the method of counting as well as the hemoglobin value have found greater variation in the count than in the hemoglobin value. In the second part of these papers the count

TABLE 1

Showing the effect of hemorrhage and plethora on the hemoglobin content, corpuscle volume and total corpuscle content of the blood. Data given in paper referred to. To make second column clear, experiment 1 blood 1 showed 44.7 per cent corpuscles: blood 2, 38.8 per cent: 38.8 = 86.8 per cent of 44.7

EXPERIMENT	IF HEMATOKRIT VALUE FOR CORPUSCLES IN ORIGINAL CAROTID BLOOD = 100, THE VALUE FOUND FOR SUBSEQUENT SAMPLE EQUALS	IF HEMOGLOBIN VALUE OF ORIGINAL CAROTID BLOOD = 100, VALUE FOUND FOR SUBSEQUENT SAMPLES EQUALS	TOTAL VOLUME OF CORPUSCLES LEFT IN BLOOD IN BODY AS DETERMINED FROM ORIGINAL SAMPLE OF BLOOD	TOTAL VOLUME OF CORPUSCLES FOUND IN BODY AS DETERMINED FROM SUBSEQUENT SAMPLES	
1	86.8	86.9	212.4	212.3	Hemorrhage = 30.8 per cent of total blood
2	95.7	95.8	239.0	238.9	Hemorrhage = 27.9 per cent of total blood
3	95.3	94.3	306.7	309.8	Hemorrhage = 28.2 per cent of total blood
.	90.0	89.6	286.7	288.1	Second hemorrhage about 9 per cent of total blood
4	92.6	92.6	387.5	387.5	Injection of 90 cc. Ringer
	86.7	86.2	270.3	272.4	Hemorrhage after the Ringer
5	96.0	96.1	817.9	817.1	Injection of 96 cc. Ringer
	93.7	93.6	586.3	588.7	Hemorrhage after the Ringer
6	96.5	96.3	974.3	975.5	Injection of 100 cc. Ringer
7	89.4	90.0	525.8	522.5	Injection of 250 cc. Ringer
	88.2	88.0	430.0	432.8	Hemorrhage after the Ringer
8	92.7	92.6	716.3	717.1	Injection of 250 cc. Ringer
	92.9	93.0	610.5	609.6	Hemorrhage after the Ringer
Average from all experiments	92.03	91.92	489.5	490.1	

was made along with the hemoglobin value. We will therefore discuss this point along with those results. I believe the above table renders it highly improbable that any large masses of corpuscles are held in the capillaries of any organ and thus the capillary blood is the

same as the blood in the large vessels. Consequently one may consider any alteration in the hemoglobin value or corpuscle mass found within a few minutes as due to the passage of fluid out of the blood to the tissues or vice versa.

EFFECTS OF ALTERATION OF BLOOD PRESSURE ON THE HEMOGLOBIN CONTENT OF THE BLOOD

Although those (Ludwig, Starling, etc.,) who have studied the flow of lymph from the thoracic duct have found that alterations of blood pressure have a great and almost controlling effect on the rate of lymph flow, the only one I have been able to find who has studied the effects of alteration of blood pressure on the blood itself is von Lesser (7). He observed a decrease in the hemoglobin content of the blood when the blood pressure was reduced by cutting the cord and a return toward normal if the cord was stimulated and the blood pressure raised. He also noticed a diminution of the hemoglobin content if the blood pressure was lowered by other factors (clamping portal vein, hemorrhage). While Cohnstein and Zuntz (8) did not make any actual pressure determinations, they followed the count during certain experimental procedures which are well known to alter the blood pressure. Thus they repeated von Lesser's work of cutting and stimulating the cord and observed similar effects. They also observed a drop in the blood count as a result of stimulating the peripheral end of the vagus and as a result of deep narcosis. Lamson (5) also observed an increase in the count as a result of asphyxia, adrenalin and other procedures known to raise the blood pressure, but he did not follow the pressure changes during his experiments.

The animals used in my own experiments were either cats or dogs. The cats were usually anesthetized with ether and urethane and the dogs with morphine and ether. However, other anesthetics (chloroform, chloral hydrate, chloretone) were used and in a number of experiments the brain was exposed under ether anesthesia and the crura cut, after which no further anesthesia was used. A continuous blood pressure tracing was always taken, usually from the carotid artery but sometimes from the femoral. A 2 per cent sodium citrate solution to which 0.5 per cent sodium chloride had been added was used in the pressure bottle. This fluid is practically isotonic with mammalian blood. The samples of blood were taken from another cannula in the other carotid or femoral artery. The blood was received into small bottles contain-

ing powdered potassium oxalate. In order to insure the same amount of oxalate being in each bottle the oxalate (usually 2 drops of a saturated solution) was put in the bottles in solution and then allowed to evaporate over an electric hot plate. About 2 cc. of blood were taken for each sample. The cannula was changed for a clean, dry one between each sample. A few drops of blood were always allowed to escape from the cannula before the sample was taken. In a few cases hirudin was used to prevent coagulation instead of oxalate and in a few other cases the blood was defibrinated. This latter is not a very satisfactory method on account of the varying number of corpuscles entangled in the fibrin.

In this series of experiments the hemoglobin was chiefly used as the index of blood change. It was estimated by converting the hemoglobin into acid hematin and comparing in a Dubosc colorimeter. Before taking the sample the bottles were rolled for about five minutes thus ensuring a uniform mixture. Shaking of the bottles is to be avoided as small bubbles are formed which do not disappear for a long time. The procedure adopted was practically the same as that described in the preceding papers (1), (2). The samples were taken by means of a 3-way glass stopcock with the cock cut diagonally. At the time of writing the above paper, I believed the idea original but somewhat similar devices are described and figured in Tigerstedt's Handbuch Physiol. Method, vol. ii. The single arm of the cock is pulled out to a point. This arm is put into the blood and filled by suction on one of the other arms. When the blood is above the cock it is closed. The point was carefully cleaned and then by turning the cock to the clean arm was allowed to discharge into a volumetric flask (usually 100 cc.) which had previously been partially filled with 0.3 per cent HCl. The same strength of acid was then run through the pipette from the clean arm and when all the blood was washed out of the pipette the flask was filled to the mark with acid. The advantage of this pipette is that it insures the same volume of blood being taken for each sample for by the washing any differences in viscosity are overcome. The arm of the pipette most used in this work held practically 1 cc. The pipette was cleaned and dried between each sample. The hematin solutions were allowed to stand for at least one hour (usually over night) before they were compared in a Dubosc colorimeter. The first sample was taken as the standard and all variations figured from it. At least three readings were taken in comparing each sample and the results averaged. Thus in all our results the hemoglobin value

of the first sample is put at 100. This figure has no reference to the total amount of hemoglobin present compared to normal. No comparisons were made between different animals but only between samples of the same animal.

The hematokrit values were obtained with the same apparatus and the same care as described previously (1). Only hirudin or defibrinated blood was used for these determinations. Only a few of these determinations were made in this series of experiments because it was found that the corpuscle volume and hemoglobin values varied so closely together (always within 1 per cent), that it was felt both determinations were unnecessary.

Since the data of this paper were gathered from simple blood pressure experiments and various ordinary procedures used to alter the pressure, the blood samples being taken at various pressures, the results may be tabulated (table 2).

In four experiments the corpuscle volume was determined as well as the hemoglobin value. The results are given in table 3.

An examination of the tables shows the following procedures have been tested:

	Experiments
Anesthetic (usually chloroform) in excess	1, 2, 4, 6, 8, 9, 12, 17, 19, 21, 25, 27, 29
Anesthetic with struggling	24
Amyl nitrite	16
Stimulation of central end of sensory nerve	8, 14, 19, 25
Division of cord in lower cervical region	5, 7, 11, 13, 20, 23
Stimulation of the cord after its section	7
Stimulation of splanchnics after section of cord	5
Asphyxia	4, 9, 10, 14, 18, 35
Hemorrhage with high and low blood pressure	22, 32
Division of both vagi	3, 4, 5, 23, 27
Stimulation of peripheral end of vagus	3, 8, 19, 22, 25
Manipulation of abdominal viscera (shock)	3, 27, 33, 34
Injection of adrenalin	2, 15, 26, 28, 30, 31

The above experiments may be divided into two groups with reference to arterial blood pressure, viz: those which raise it (struggling, first stages of asphyxia, stimulation of central end of sensory nerve, stimulation of cord or splanchnic nerve, division of both vagi, adrenalin) and those that lower it (excess of anesthetic, amyl nitrite, last stages of asphyxia, division of cord, stimulation of peripheral vagus, manipulation of viscera shock!). It will be noticed that all those procedures which cause a rise of blood pressure lead to an increase in the hemo-

TABLE 2

EXPERIMENT	ANIMAL		TIME	BLOOD PRESSURE, MILLIMETERS MERCURY	HEMOGLOBIN
1	Cat	First sample taken	10.55	126	100.0
		Chloroform pressed—pressure fell to 45, then as anesthetic relaxed rose	10.59	65–80	93.0
		Chloroform removed 10.58	11.02½	126	98.0
2	Dog	First sample taken	10.50	120	100.0
		From 10.50 to 11.20 did experiment on respiration. Pressure fairly constant all time	11.42	130	100.0
		Injected 10 cc. dilute adrenalin at 11.50	11.51½	200	103.0
			11.55	130	100.5
		Chloroform commencing 11.56	12.02	60	92.0
3	Dog (Pup)	First sample taken at	10.12	120	100.0
		Cut right vagus at 10.15½, left at 10.17	10.26	170	114.0
		Stimulated peripheral vagus 10.30–10.34	10.34½	90–100	87.0
		Ceased stimulation; pressure rose	10.41	160	116.0
		Opened abdomen and manipulated viscera	11.10	110	93.0
4	Dog 7.4 kilo	Morphia—Used artificial respiration. First sample taken	9.27	120	100.0
		Cut left vagus at 9.29	9.42	164	103.3
		Stopped respiration at 9.45 and cut right vagus	9.54	200	107.0
		Pressure rose to nearly 300 and then gradually fell to nearly zero. Did abdominal massage. Had to open thorax and massage heart	10.06½	100–10	89.0
		Pressure stayed fairly constant	10.24	100	98.0
		Chloroform pressure fell	10.31	20	80.0
5	Dog	First sample at	10.00	140	100.0
		Cut both vagi; no effect on pressure, more anesthetic	10.10	130	98.0
		Cord cut in lower cervical region	10.45	78	93.7
		Tried to stimulate cord. Opened abdomen and stimulated splanchnic	10.57	130	99.3
		Removed stimulus; pressure gradually fell	11.03	60	93.0

TABLE 2—Continued

EXPERIMENT	ANIMAL		TIME	BLOOD PRESSURE, MILLIMETERS MERCURY	HEMOGLOBIN
6	Dog	First sample at	11.31	120	100.0
		Chloroform pressed	11.35	50	97.0
		Chloroform removed at 11.34	11.40	110	99.0
7	Cat	First sample	10.31	150	100.0
		Cut cord	10.56	40	76.9
		Stimulated cord; rise to 100, then gradually fell	11.01	100–80	83.3
		Stimulation stopped at 11.01	11.05	45	81.0
8	Cat	Under ether cut the crura. No more anesthetic			
		First sample	3.11	200	100.0
		Stimulated central end of sciatic	3.17	280	108.0
		Stopped stimulus 3.17	3.22	190	104.0
		Vagus cut; peripheral end stimulated 1 m.	3.29	110	100.0
		Chloroform pressed	3.39	45	93.0
9	Cat	First sample	10.20	150	100.0
		Chloroform pressed	10.30	53	95.0
		Chloroform removed 10.30	10.38	135	98.5
		At 10.45 clamped trachea and cut vagi. Pressure did not rise much but gradually fell	10.56	55	83.0
10	Cat	First sample taken at	10.30	110	100.0
		Trachea clamped and then opened; pressure rose to 195 where it stayed for 1 minute and then fell to 80	10.50	195–80	102.0
11	Dog	First sample taken at	10.30	90	100.0
		Cut cord about 11.10	11.15	40	90.0
12	Cat	First sample taken at	10.05	116	100.0
		Chloroform pressed	10.10	30	96.5
		Chloroform removed at 10.10	10.17	150	102.0
13	Dog	First sample taken at	10.40	100	100.0
		Cut cord about 11.10	11.30	60	96.5

TABLE 2—Continued

EXPERIMENT	ANIMAL		TIME	BLOOD PRESSURE, MILLIMETERS MERCURY	HEMOGLOBIN
14	Cat	Cut crura under ether. Artificial respiration most of time. First sample....	3.10	130	100.0
		3.11 stopped respiration, pressure rose with large variations to 210, most time about 150.....	3.15	210	103.0
		Respiration commenced again. Gradual fall of pressure till at.....	3.30	70	80.0
		Stimulated central end of sciatic 2½ minutes.....	3.33	90	89.0
		Clamped trachea again, pressure rose from 70 to 160, then fell with variation due to giving a little air.....	3.53	36	84.0
15	Cat	Ether chloretone. First sample.....	10.53	30	100.0
		Injected adrenalin; pressure rose to 160 and then gradually fell.....	11.01	110	112.0
		Pressure gradually fell.....	11.23	20	104.5
16	Cat	Chloroform; crura cut. First sample....	10.22	130	100.0
		Amyl nitrite.....	11.00	75	93.0
17	Cat	First sample at.....	9.30	175	100.0
		A. C. E. pressure fell rapidly to.....	9.45	35	97.0
		Artificial respiration. Rise again.....	9.57	173	100.5
18	Cat	First sample.....	4.00	95	100.0
		Clamped trachea, pressure rose to 123 and then gradually fell.....	4.05	45	87.0
19	Dog	Morphia; artificial respiration, cut one vagus. First sample.....	10.25	150	100.0
		Stimulated vagus 10.28–10.36, average pressure 2 minutes.....	10.30	110	98.0
		After pressure recovered.....	10.35	145	100.0
		Stimulated central end of sciatic 10.38 to 10.40.....	10.40	240	101.0
		After stopping stimulus.....	10.45	175	100.0
		Chloroform pressed.....	11.00	60	95.0
		Chloroform removed at 11.00.....	11.10	86	98.0
20	Dog	First sample.....	10.45	100	100.0
		Cut cord about 11.20.....	11.30	45	98.0

TABLE 2—Continued

EXPERIMENT	ANIMAL		TIME	BLOOD PRESSURE, MILLIMETERS MERCURY	HEMOGLOBIN
21	Cat	First sample	10.55	115	100.0
		Chloroform pressed	11.00	80	93.5
		Chloroform removed at 11.00	11.05	115	98.0
22	Dog 6.7 kilo	Cut left vagus. First sample	10.00	170	100.0
		Stimulated vagus 1½ minutes 10.10–10.07½	10.08	100	91.0
		After stopping stimulation	10.12	180	99.0
		Stimulated central sciatic and did experiment on asphyxia. Samples lost	11.00	100	100.0
		A. C. E. pressed; pressure fell	11.13	32	87.0
		Bled 115 cc. pressure fell to about 12 and then gradually rose	12.10	56	88.0
		Reinjected blood at 12.11	12.15	80	97.0
		Bled 100 cc., pressure fell	12.25	30	87.0
23	Dog	First sample	10.04	109	100.0
		Cut both vagi at 10.06	10.11	141	105.0
		Cut cord cervical region about 10.45	11.00	53½	87.0
24	Cat	First sample	9.44	118	100.0
		Chloroform struggling	9.50	130	102.0
		Got animal quiet	10.00	110	100.0
25	Dog	Divided left vagus and sciatic nerve. First sample	10.12	110	100.0
		Vagus stimulated 10.16–10.19	10.19	80	98.0
		Stimulated central end of sciatic 10.22–10.25	10.25	140	104.0
		Stopped stimulation at 10.25	10.31	110	101.0
		Pressed chloroform	10.36	50	100.0
26	Dog	First sample	4.00	100	100.0
		Adrenalin injected	4.03	180	114.0
		Pressure gradually fell	4.10	60	108.0
		Pressure gradually rose	4.20	90	100.0
27	Cat	Chloroform; crura cut; natural respiration. First sample	8.50	136	100.0
		Divided both vagi at 8.53	8.58	200	103.0
		Chloroform pressed; pressure went to 20	9.17	30	87.0
		Chloroform removed at 9.17; hard time to resuscitate	9.24	130	88.0
		Opened abdomen	9.37	58	84.0
		Pressure kept falling	9.55	25	83.0

TABLE 2—Concluded

EXPERIMENT	ANIMAL		TIME	BLOOD PRESSURE, MILLIMETERS MERCURY	HEMOGLOBIN
28	Dog	First sample	4.05	110	100.0
		Adrenalin injected 4.08	4.10	160	103.0
29	Cat	First sample	9.00	128	100.0
		Chloroform pressed	9.10	50	98.0
		Chloroform removed at 9.10	9.20	126	102.0
30	Dog	First sample	3.50	130	100.0
		Adrenalin injected	3.55	200	106.0
31	Dog	First sample	3.50	104	100.0
		Injected adrenalin at 3.54	3.56	194	102.0
		Pressure gradually fell	4.05	94	103.0
32	Dog 8 kilo	First sample	1.30	122	100.0
		Pressed A. C. E	1.44	80	99.0
		Bled 110 cc. at 1.44; removed anesthetic, pressure gradually rose	1.56	110	97.5
		Reinjected 75 cc. blood at 1.58	2.09	115	101.0
		Bled 75 cc. at 2.09	2.22	40	90.0
33	Cat	First sample	2.25	110	100.0
		Did abdominal operation; "shock"	3.46	70	74.0
34	Cat	First sample	3.01	136	100.0
		Did abdominal operation; "shock"	4.05	50	90.0
35	Cat	First sample at	10.44	150	100.0
		Did class demonstration. Clamped trachea, took sample near end of asphyxia	11.50	50	95.8

globin content of the blood and all those procedures which cause a fall in blood pressure lead to a drop in the hemoglobin content. Since these results are obtained within a few minutes they can be explained, I believe, only on the hypothesis that the fluid content of the blood has altered—a rise of pressure forcing fluid out of blood into the tissue spaces and a fall of pressure leading to a passage of the fluid back from the tissue spaces into the blood. The course of the blood pressure

TABLE 3

Showing the comparison between corpuscle volume and hemoglobin value in bloods of certain experiments. In experiments 4 and 5 the values are taken from samples of blood that had been defibrinated. Hence the values for hemoglobin do not exactly agree with those given in the table of experiments taken from samples of oxalate bloods

EXPERIMENT	BLOODS	VOLUME OF CORPUSCLES	CHANGE IN VOLUME OF CORPUSCLES TAKING ORIGINAL VOLUME AS 100	CHANGE IN THE HEMOGLOBIN VALUE TAKING HEMOGLOBIN IN FIRST BLOOD AS 100
4	1	47.7	100.0	100.0
	2	49.75	104.2	105.0
5	1	32.6	100.0	100.0
	2	32.0	98.1	98.0
	3	31.6	96.9	96.0
	4	32.6	100.0	100.0
29	1	46.3	100.0	100.0
	2	45.1	97.4	98.0
35	1	41.7	100.0	100.0
	2	39.5	95.7	95.8
Average from all bloods.....			99.23	99.28

and the hemoglobin during asphyxia are especially interesting in this regard. If the samples are taken when the blood pressure is high, the hemoglobin content of the blood is increased but if one waits until the final stages of asphyxia when the pressure is low then the hemoglobin content is reduced. Also the effect of hemorrhage on the blood is interesting. Lamson (5) calls attention to the fact that hemorrhage does not always lead to a decrease in blood count. Whether you get an increase or a decrease in the hemoglobin value of the blood as a result of hemorrhage depends on how the hemorrhage affects the blood pressure. If you first decrease the blood pressure and bleed when the pressure is low and then if the blood pressure recovers, you will find little alteration in the hemoglobin value. Experiments 22 and 32 show such cases. The passage of fluid out of the blood takes place, no doubt, in the capillaries. As Bayliss and Starling (9) have pointed out, because one gets an alteration of arterial pressure it does not necessarily mean an alteration of capillary pressure in the same direction. Thus Bayliss and Starling find a rise of capillary pressure in the liver on vagus stim-

ulation. Seeing that the liver capillaries are the most permeable in the body one might not have been surprised to obtain a concentration of the blood on this procedure. Both Cohnstein and Zuntz and I find a diminished hemoglobin content showing the effect in other organs more than overcame the effect in the liver. The same remarks apply to asphyxia where Bayliss and Starling report a rise of capillary pressure in the liver till death.

To me the most surprising thing in their results is the rapidity with which alterations of pressure affect the fluid content of the blood. During all these experiments I have not found an exception to the rule that a rise of pressure leads to an increase in the hemoglobin content of the blood and vice versa. The rise in the hemoglobin after adrenalin injection occurs as promptly as with other procedures. After the pressure begins to fall, however, the hemoglobin seems to keep up for a time although the pressure has returned to normal. Adrenalin is destroyed chiefly in the liver (10) and probably affects the capillaries of this organ which as we shall see probably plays the greatest part in these results. If only enough adrenalin is used to affect the pressure for a short time, the return of pressure to normal is rapidly followed by a return of the hemoglobin to normal. Between different animals there is considerable difference in the quantitative amount of change as a result of the same procedure. Two other factors (at least) besides the blood pressure have to be considered—permeability of the capillary wall and difference in colloidal concentration between the plasma and tissue fluid. The difference in quantitative results may be seen if we tabulate the results with chloroform for the cat.

Experiment 1 pressure went from 126 to 65 = 61 mm. hemoglobin dropped 7 per cent.
Experiment 8 pressure went from 190 to 45 = 145 mm. hemoglobin dropped 11 per cent.
Experiment 9 pressure went from 150 to 53 = 79 mm. hemoglobin dropped 5 per cent.
Experiment 12 pressure went from 116 to 30 = 86 mm. hemoglobin dropped 3.5 per cent.
Experiment 17 pressure went from 175 to 35 = 140 mm. hemoglobin dropped 3(8) per cent.
Experiment 21 pressure went from 115 to 80 = 35 mm. hemoglobin dropped 6.5 per cent.
Experiment 27 pressure went from 200 to 30 = 170 mm. hemoglobin dropped 16 per cent.
Experiment 29 pressure went from 128 to 50 = 78 mm. hemoglobin dropped 2 per cent.

(Note in experiment 17 the recovery value was 8 per cent above low value)

No doubt part of the difference in the results is due to differences in the length of time during which the blood pressure was kept altered. The pulse pressure also probably has an influence although my results are not clear on this point. Another factor to be considered with chloroform is the increased osmotic pressure of the blood due to the absorbed anesthetic (11). This would tend to produce a condition of hydraemic plethora. While different amounts of anesthetic in the blood would also be a factor causing quantitative differences in different animals in the above table, yet I believe it is not the main factor since the omsotic pressure of the lymph is also increased with increased anesthetic and similar differences are found for other procedures where the anesthetic was kept uniform, e.g., section of cord, stimulation of vagus. I believe the main difference in the quantitative results obtained with different animals lies in the character of the blood and in the permeability of the capillary walls. In the blood the main difference would be in its colloidal content. The difference in the colloidal content of the blood and tissue fluid seems to be the factor pulling fluid back from the tissue spaces to the blood (12), (2), and hence different animals would respond quantitatively differently to a similar drop in blood pressure. The same considerations would no doubt play a part in the filtering out process when the blood pressure is increased. Experiments in which the blood pressure is maintained at different levels over similar periods of time together with an examination of the colloidal content of the blood are being undertaken.

The permeability of the capillary wall is no doubt also a factor in the quantitative results from alteration of pressure. Young animals seemed to react with greater changes than old ones. The capillaries in various organs are known to have a different permeability. Thus Starling (13) showed that the increased flow of lymph caused by a rise of blood pressure came from the liver and intestines. The liver capillaries are the most permeable. The lymph coming from the liver has more protein in it than that from other organs. In order to produce an increased flow of lymph from the limbs the pressure has to be greatly increased or the capillaries damaged. Cohnstein and Zuntz, however, observed an increase in count in the blood in the leg on clamping the femoral vein for a few minutes. Lamson believes the liver is the only organ involved in the results he has described. A number of experiments have been made dealing with the permeability in different organs. The results are as yet incomplete. Thus after obtaining the results recorded for experiments 15, 17, 21, 24, 26, 29, 30 and 31

the abdomen was opened and everything in the liver pedicle except the portal vein clamped. The same procedure (chloroform or adrenalin) was then tried again. In experiments 17 and 31 there was practically no effect on repeating the experiment with the hepatic artery clamped. In three others (15, 24, 30) there was a distinct effect but not as great as before while in the other three (21,26, 29) the effect was nearly as great as before clamping the pedicle. It is evident the capillary pressure in the various organs must be followed during these procedures. While the liver, owing to the greater permeability of its capillaries, is probably the place where the effects of altered blood pressure are most felt, yet I believe other organs play a part. These problems are being further investigated.

RESULTS

The hemoglobin content of the blood is rapidly modified by any alteration in blood pressure, a rise of pressure leading to an increase of hemoglobin and a fall to a decrease. These results can only be explained by the increased pressure forcing fluid out of the blood to the tissue spaces and the passage of fluid back from the tissue spaces to the blood when the pressure is lowered.

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FACTORS INFLUENCING THE INTERCHANGE OF FLUID BETWEEN BLOOD AND TISSUE SPACES

II. Muscular Activity

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In exercise there are two factors which might lead to an altered concentration of the blood, viz., blood pressure and muscular activity. The first of these factors has been dealt with in part I of this series (1). In this paper we will deal with the effects of muscular contraction on the blood. Several facts are known about muscle which would lead one to believe that muscle contraction would tend to concentrate the blood. Thus judging from the amount of water it will take up from salt solutions, fatigued muscle has a higher osmotic pressure than resting muscle (2), (3). The freezing point of fatigued muscle is lower than that of resting muscle but if the circulation be kept going through the muscle while it is being stimulated, there is little alteration in the freezing points (4). The specific gravity of muscle decreases during activity while its weight increases (5), (6), (7). The water content of frogs' muscle was observed to increase as a result of contraction produced reflexly after strychnine injection or as a result of nerve stimulation (8). Ranke observed an average increase of about 1.7 per cent in the water of the stimulated muscle. Barcroft and Kato (6) dealt with the gastrocnemius muscle of the dog and determined the oxygen consumption, rate of blood flow as well as the concentration of the blood. They stimulated 0.2 of every second for fifteen minutes and observed an increase of about 19 per cent of the muscle's weight. The specific gravity of the muscle decreased from 1070 to 1061. Barcroft and Kato give only their results and not the figures for the blood changes. We have made some determinations on the water content of resting and active muscle to see if the height of blood pressure had any influence in the results. Some determinations were first made on the gastrocnemius of the frog. In the first experiments after single pithing we divided one sciatic nerve and stimu-

lated it for ten minutes with induction shocks. Our figures resemble those given by Back, Cogan and Towers (7). We found as they did that occasionally the stimulated muscle showed less water than the resting one. In order to see if interfering with the blood supply had any effect we divided the nerve on one side and left it without stimulation for ten minutes. In nine experiments on the frog we found that six muscles with the nerve cut contained more water and three contained less. A series of experiments was also done on the frog in which both sciatics were cut and then one stimulated. Here again we observed similar variations. These results are probably due to the varying amount of water in the frog's muscle to commence with or to the inability to get rid of adhering lymph equally on both sides. We then decided to try the muscle of the cat and divide the experiments into two classes—those in which the blood pressure was low and those in which it was high. The muscles used were those of the forearm. The nerves of the brachial plexus were cut on both sides and then one arm tetanized for two minutes. After stimulation, both legs were massaged and then the muscles exposed as rapidly as possible and a small piece taken, weighed and dried at 105° to a constant temperature. The pieces from the two sides were taken from as near the same part of the muscle as possible. The results are as follows:

	BLOOD PRESSURE	WATER STIMULATED MUSCLE	WATER RESTING MUSCLE	DIFFERENCE
		per cent	*per cent*	*per cent*
1	36	74.600	74.908	−0.308
2	50	74.575	73.827	+0.748
3	50	75.535	75.180	+0.355
4	45	79.438	76.076	+3.362
5	35	75.089	74.387	+0.702
6	170	76.837	75.244	+1.593
7	110	75.716	75.248	+0.468
8	150	77.109	76.100	+1.009
9	172	75.375	75.480	−0.105
10	150	76.919	75.273	+1.046
11	100	77.860	74.527	+3.333
12		76.525	76.152	+0.373
13		74.763	73.478	+1.285
14		73.420	72.486	+0.934
15		73.969	74.200	−0.231
16		77.824	75.605	+2.219
Average.....		75.972	74.886	+1.086

It will be noticed that in thirteen out of the sixteen experiments there is an increase of the water content of the muscle but in three there is a decrease. The figures on the whole, however, show a decided tendency for the water to increase as a result of stimulation. No relationship to the blood pressure could be made out. Experiments 12 to 16 were taken from animals used for other experiments in which the blood pressure was not recorded but they were probably low. In none of our experiments did we get as large an increase as that found by Barcroft and Kato, probably because the stimulation was much shorter. From the above work, however, it is evident that if any considerable mass of muscles goes into activity there would probably be considerable effect on the blood.

In examining the effects of muscular contraction on the blood we tried to put as large a mass of muscles into contraction leaving the remainder of the animal as normal as possible. For this purpose we sectioned both sciatic nerves just as they entered the thigh. The peripheral ends were pulled through Sherrington electrodes and connected in circuit to the poles of a secondary coil. A continuous carotid pressure tracing was always taken during the experiment. The samples of blood were taken from the other carotid artery. The same technique was used as described in the first part of this work. In the blood, in most cases, the blood count was made as well as the hemoglobin estimated. The count was made using a Thoma Zeiss counter and isotonic saline as the diluting fluid. The same pipette and counter were used throughout the work. The hemoglobin was estimated with a Dubosc colorimeter after converting it to acid hematin as described in part I. The counts were all made by the same person (E. T. H.) and the hemoglobin determinations made by another (F. H. S.).

The animals used were chiefly cats. They were anesthetized with ether and urethane. The few dogs used were anesthetized with morphine and ether. After the animals were anesthetized, the cannulae were inserted, the nerves prepared and the electrodes attached. A first or normal blood sample was taken. Then the muscles were tetanized, usually continuously but sometimes with short intervals of pause for a definite time. A sample of blood was usually taken at the end of one or two minutes' stimulation and then the stimulation continued for seven or eight minutes longer when a third sample was taken. A fourth sample was taken ten to twenty minutes after the end of stimulation. The first sample was taken from two to five minutes before the stimulation commenced. The muscles were held in tetanus by the

stimulation. In the longer periods of stimulation short intervals of rest were allowed but the rest period was only a small fraction of the total time. The blood pressure remained fairly constant in all the experiments. Shortly after commencing stimulation there is usually a small rise of pressure due probably to the heated blood reaching and affecting the heart but there is not enough pressure change to account for the results described. A protocol of one complete experiment may be given and then the remaining experiments tabulated.

Experiment 1. Cat, November 1, 1916. Ether urethane. Cannulae in carotids. Sciatics exposed and cut. First sample taken at 10.54; blood pressure 104. Stimulation from 10.57 to 11.06. At 10.58 took second sample; blood pressure 120. The pressure dropped gradually till at 11.06 when third sample was taken it was 110. At 11.16 took fourth sample; pressure 108. The counts were blood 1 = 7,680,000; blood 2 = 7,928,000, (equals an increase of 3 per cent on blood 1); blood 3 = 8,368,000 (equals an increase of 9 per cent on blood 1); blood 4 = 7,684,000 (same as blood 1). The values found for the hemoglobin were as follows: With colorimeter 10 of 1 = 9.8, 9.9, 9.8, 9.8 of 2. Therefore blood 2 has 102 per cent hemoglobin. With blood 3, 9.6, 9.5, 9.5, 9.5 were equivalent to 10 of blood 1. Therefore blood 3 has 105 per cent hemoglobin. With blood 4, 10.1, 10.0, 10.0, 10.0 equalled 10 of blood 1. Therefore blood 4 has 100 per cent hemoglobin.

Since it is the relative figures that are important in the following table only the count for blood 1 is given. This is taken as the standard and the increased or decreased percentages given. A plus (+) sign means an increase and a minus (−) sign a decrease. The same is true of the hemoglobin where the first blood is always figured as 100. In some cases the stimulation was not kept up for more than two minutes. The blank spaces in the table indicate that the samples were not taken and indicate whether the stimulation was short or long.

An examination of the following table shows that the contraction of the leg muscles leads to a concentration of the blood. This is due, no doubt, to the passage of fluid from the blood to the muscles as found by Ranke and by Barcroft and Kato. These results are comparable in many cases to those found as the result of exercise in man as recorded by v. Willebrand (9), Hawk (10) and Schneider and Havens (11). It will also be noticed that the longer period of stimulation does not always lead to a greater effect than the shorter. In fact, in about half the cases the result of contraction for ten minutes is less than that for two minutes. This is no doubt due to the passage of the products of activity back into the blood and thus tending to restore the osmotic equilibrium. [Ryfel (12) has shown that lactic acid may be detected in

the urine in large amounts after exercise (818 mgms. in one case after running five minutes).] Ten to fifteen minutes after the stoppage of contraction the blood in most cases has returned nearly to normal. In some cases the after-effect is a decrease in the concentration of the blood. This is probably also due to the increased amount of waste product in the blood. The increased osmotic pressure of the blood would affect all organs in the body and thus tend to dilute the blood.

TABLE I

Showing the effects of contraction of the leg muscles on the concentration of the blood

EXPERIMENT	ANIMAL	FIRST BLOOD COUNT	ALTERATION IN BLOOD AS A RESULT OF SHORT CONTRACTION (1 TO 3 MINUTES)		ALTERATION IN BLOOD AS A RESULT OF LONGER CONTRACTION (5 TO 11 MINUTES)		ALTERATION IN BLOOD 10 TO 15 MINUTES AFTER END OF CONTRACTION	
			Count	Hemoglobin	Count	Hemoglobin	Count	Hemoglobin
1	Cat	7,680,000	+3	+2	+9	+5	0	0
2	Dog	7,456,000	+4.8	+3	−2	+3	−7	−2
3	Cat	8,820,000	+11	+5				−4
4	Cat	8,654,000	+1	+6				−5
5	Cat	8,702,000			+8	+4	0	−1
6	Cat	7,472,000	+14	Lost	+1.3	+2		
7	Cat			+5		−2		−10
8	Cat			+2		+8		0
9	Cat	6,288,000	+15	+4		+8		+2
10	Cat	7,720,000	+2	0	+7	+5		
11	Cat	8,080,000	+13		+9		+2	
12	Cat	9,520,000	+5	+2	−3.5	−4		
13	Cat	9,304,000	+12	+1	+3.5	+3	−19	−2
14	Cat	9,144,000	+8	+2			+9	+1
15	Cat	9,020,000	+2	+5				
16	Dog	9,360,000	+15	Lost	+15	+5	+2	+2
17	Cat	8,230,000	+12	+2			+10	+2
Average			+8.4	+3	+5.2	+3.3	−0.4	−1.6

It will be remembered that those (see above) who have studied the effects of exercise on the blood of man found greater effects from short exercise (100 yards) than from the longer runs.

There is one other matter to which we wish to call attention and that is the relation of the blood count to the hemoglobin values. If one examines the table one notices that in practically every instance the hemoglobin value and the count vary in the same direction but in many cases in quite different amounts. A good many others who have

followed the hemoglobin content and the blood count through a series of experiments have found similar results. (See Krehl's Clinical Pathology, Inagaki (13), Lamson (14)). Inagaki explains this on Bohr's hypothesis of different hemoglobins. We believe this hypothesis has been disproved by the work of Barcroft and his pupils. Lamson believes the liver has the function of splitting the corpuscles or storing them. We believe the variation found in the hemoglobin content and count has no physiological significance but is due to the errors in the estimations. An examination of the table shows that in experiments 1, 10, 12 the results agree right through within small limits of error. In experiment 13 (and most others) for example, the count and hemoglobin do not vary in such narrow limits. It may only be a coincidence but in every experiment except two (14 and 17) where the blood count and hemoglobin were made on more than two samples, the hemoglobin value and the count agreed at some point. If this variation were not due to technical errors it is hard to understand why in a series of experiments in which the same procedures were followed, this point of agreement should sometimes be on the second blood, sometimes on the third, sometimes on the fourth and sometimes not at all. Also it it to be noted that while in most cases the count varied more than the hemoglobin in a few cases the greater variation lay with the hemoglobin. We are conducting a series of experiments trying to find the cause of this lack of agreement between the count and the hemoglobin values.

RESULTS

There is an increase in the water content of muscle as a result of contraction if the blood is circulating through it.

There is an increase in the hemoglobin content and blood count as a result of muscular contraction. This is due to the passage of fluid from the blood to the muscles.

In some experiments the alterations found for the hemoglobin and for the blood count are not exactly the same. We believe this is due to technical errors and has no physiological significance.

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ON THE PRESENCE OF ALBUMOSES IN THE TISSUES AND IN THE BLOOD, WITH SPECIAL REFERENCE TO THEIR OCCURRENCE IN THE GASTRO-INTESTINAL MUCOSA[1]

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It will be admitted that it is a matter of physiological importance if it can be established (1) that albumoses are present in various organs of the body as integral components of their cells; (2) that the absorbing mucosa of the stomach and small intestine contain these substances in larger amount than other tissue, especially during the digestive period, and (3) that the plasma of the blood, the transporting agent of the body, carries these substances from the stomach and intestines to other tissues. A study of these questions which, as is well known, are not new, was forced upon us when we undertook to isolate certain active constituents of the mucosa of the stomach and the small intestine. It was not difficult to prepare from this tissue a water-soluble powder which showed great physiological activity in stimulating an intestinal or uterine strip or in causing the pancreas to secrete freely. Always such powders, although entirely free from coagulable proteids, gave both the Pauly and the biuret reactions. We would note here that one of our preparations from the intestinal mucosa of the pig, which caused almost maximal contractions of the virgin guinea pig's uterus in the proportion of 1: 1,000,000 and which also caused a distinct rise of blood pressure (cat), furnished a colorless aqueous solution which in these respects, as well in its chemical reactions (biuret and Pauly reactions), behavior towards polarized light and towards saturation with ammonium sulphate, was strictly comparable to a somewhat diluted aqueous extra of the pituitary gland. This striking analogy led to a more careful study of the biuret-yielding substance and its identification as a secondary albumose.

That a stimulant for the intestinal musculature can be extracted from the cells of the gastric and intestinal mucosa was first shown by

[1] This investigation was aided by a grant from The Rockefeller Institute for Medical Research.

Enriquez and Hallion (1). It is not our purpose now to report on or analyze the great number of investigations that deal with the "peristaltic hormones" (2) that have been isolated from many organs of the body, but we wish to emphasize the fact that it is not difficult to prepare an extract of the intestinal mucosa, like that referred to above, which is highly active in the proportion of 1: 1,000,000. Inasmuch as the active motiline can be present only in small amount in this extract, we may safely predict that when once it has been isolated as a chemical individual it will be found to be no less active than β-imidoazolylethylamine or than the uterine stimulant of the pituitary gland. The thought naturally arises that this powerful motor constituent of the pituitary body is possibly identical with that shown to be present in the gastric and intestinal mucosa and elsewhere in the body. The great activity of only partially purified extracts of the gastric and intestinal mucosa, which still contain a preponderance of albumoses, amino acids and inorganic salts, certainly justifies us in raising this question. We hope to be able at a later date to give something more tangible than a hypothesis on this point.[2]

ALBUMOSE IN THE GASTRIC AND INTESTINAL MUCOSA

As to the presence of albumoses in the gastric and intestinal mucosa, it is well known that Hofmeister, one of the earlier investigators in this field, maintained that "peptone" is present in these tissues. As far as is known to us, the rigid proof for such an assertion, which consists in the actual isolation of an albumose or peptone from the gastric and intestinal mucosa, was not given by Hofmeister or by his pupils. Indeed, with a few exceptions (Nolf, Asher and their pupils), physiologists and bio-chemists are inclined to the opinion that the proteids of our food are absorbed only in the form of the ultimate end products of digestion—the amino acids, although the possibility that lower polypeptids may also pass into the blood is admitted.

[2] We find that Köhler (Organextracte als Wehenmittel, Zentralb. f. Gynäkologie, 1915, 891), while offering no opinion as to the identity of the substances concerned, has suggested that an extract of some organ or organs other than the hypophysis might be used instead of the latter as an oxytocic in therapeutics. In our opinion, the mucosa of the pig's stomach, from which, as already stated, a preparation that raises the blood pressure and stimulates the uterus and the intestine may readily be prepared, should furnish a substitute for the costly hypophysis preparations. Further research must determine whether this suggestion is commercially feasible.

Some have no doubt been willing to admit the taking up by the intestinal and possibly by the gastric mucosa of albumoses and peptones (the biuret-yielding polypeptids) but finding no evidence that the plasma of the portal blood is a carrier of such biuret-yielding polypeptids, these authors have fallen back on the supposition that these compounds are broken down to abiuret products, amino acids and lower polypeptids, in the mucosa itself.

Our method can be described here in outline only. Many details of the operations will be self-evident to experienced workers. The treatment was the same with both the gastric and intestinal mucosa. The description here given applies specifically to the upper portion (three to four feet in length) of the small intestine of the pig, but is equally serviceable in working up the mucosa of the stomach. The intestines were brought quite fresh, many being still warm, from a slaughter house in close proximity to the laboratory. They were immediately slit open and thoroughly cleaned by agitation under a current of water until freed from adherent mucus and bile, after which the mucous membrane was scraped away with a glass plate.

The scrapings were weighed and put into large flasks and 95 per cent alcohol was introduced in small portions at a time, the flask being continually shaken, until the mixture contained from 45 to 50 per cent of alcohol. For purposes of calculation, one gram of gut scrapings was called the equivalent of one cubic centimeter of water, and in most of our work we added one liter of 95 per cent alcohol to each kilogram of gut scrapings.

The flasks, after thorough admixture of their contents, were then attached to reflux condensers, the body of the flask in each case being submerged in water, and the contents were kept at a lively boil for an hour.[3] The coagulated material was now removed and pressed to a hard cake in a small wine press, and the clear or slightly turbid alcoholic filtrate, which contains our biuret substances, was concentrated to a small volume on the water bath with the assistance of an electric fan. When the alcohol and much of the water had been so far removed that the remaining solution had the consistency of an easily flowing syrup, it was transferred to an Erlenmeyer flask and while it was still warm and freely mobile, 95 per cent alcohol was added with a pipette in small quantities at a time with thorough agitation, so that the precipitation took place only very slowly. It is often advisable to return the flask to the water bath from time to time during the early stages of the precipitation.

When the alcoholic strength of the mixture had reached from 50 to 60 per cent at the highest the flask was set aside until the precipitate had settled out and a clear fluid could be poured off. The precipitate was then transferred to plate filters, drained at the pump, washed with 50 to 60 per cent alcohol and transferred to a large receptacle in which it could be exposed to the extracting action of 50

[3] As is pointed out elsewhere in this paper, a much shorter time of boiling, perhaps fifteen minutes, will suffice to dissolve out the albumoses. The above period of one hour was adhered to in experiments whose primary object was a large yield of secretine.

to 60 per cent alcohol; after a time the supernatant fluid was drawn off and worked up with the main filtrate in the manner to be described.

The alcoholic filtrates were again concentrated on the water bath under a rapidly revolving electric fan until a mobile syrup was once more obtained. This was transferred, while still hot, to large Erlenmeyer flasks and absolute alcohol was added in very small quantities at a time with thorough agitation and occasional warming until a voluminous, crumbly precipitate had settled out. At this point the alcoholic strength of the mixture had probably attained a value of 85 per cent or more, and the further addition of absolute alcohol yielded but little more of the precipitate. Nevertheless, it has been our practice to add absolute alcohol until the precipitation is finished, and in order to precipitate the biuret substance as completely as possible we added much ether at this stage. The flasks were now allowed to stand for a number of hours, or until the clear supernatant alcohol or alcohol-ether mixture could be removed with a siphon and the crumbly, though often partly sticky, precipitate could be collected on flat filters at the pump. No difficulty was encountered in disintegrating and thoroughly washing the precipitate with strong alcohol and finally with a mixture of absolute alcohol and ether. After being washed free of all fatty and other substances which are soluble in absolute alcohol and ether,[4] the precipitate was dried *in vacuo* over sulphuric acid and ground up to a fine powder.

An aqueous solution of this powder gives the biuret reaction with great intensity. In order to remove all traces of coagulable proteid the powder was dissolved in water and a 40 per cent solution of lead acetate was added until no more precipitate fell out. To the filtrate basic lead acetate (30 per cent) was added until precipitation was complete, care being taken to avoid a large excess of this reagent. The precipitate was removed at the pump, to the filtrate 95 per cent alcohol was added until the solution attained a strength of 50 per cent and the lead was precipitated by the addition of sulphuric acid in 50 per cent alcohol. This precipitation is so complete in 50 per cent alcoholic medium that hydrogen sulphide no longer produces a blackening. After the removal of the lead sulphate by filtration and of the alcohol by evaporation, the solution was found to be free of native proteids. At one time we added uranyl acetate to the neutralized solution at this point in order to allay any suspicion that an alcohol-soluble proteid might have escaped the action of the above named precipitants, but the results were unaltered—the filtrate after removal of the uranium still showed the presence of proteose as before. The use of mercuric chloride as a precipitant at this point, that is, when it is added to the acidulated alcohol-water solution, also leaves our proteose in solution, again showing that it must be a substance of the character of a secondary albumose.

Further purification of the product (removal of secretine, "motiline," amino acids and primary albumoses) was effected by the use of phosphotungstic acid, mercuric chloride, silver nitrate and repeated precipitation of the final product in alcohol of varying strengths. Phosphotungstic acid (1: 1) was added directly to the alcoholic, lead-free filtrate until no further precipitate settled out. This precipitation occurs in very dilute solutions, and in such solutions only the less soluble phosphotungstates, as those of the albumoses, fall out in relatively large

[4] U. S. P. ether was used for this purpose.

amounts. The precipitated phosphotungstates were collected at the filter pump, washed as well as possible and pressed to a hard cake. The cake was ground up in a mortar with dilute sodium hydroxide solution until the entire cake, with the exception of an inconsiderable residue, had passed into solution. The solution should be neutral or faintly acid. In case it shows a slight turbidity a drop of alkali will at once clear it up. The phosphotungstates were now precipitated by the addition of dilute sulphuric acid, again collected in the form of a hard cake, and the process of solution in dilute alkali and reprecipitation with sulphuric acid was repeated one or more times.

The cake was then ground up in a mortar with dilute sodium hydroxide until a clear neutral or only faintly alkaline solution was obtained. Saturated barium hydroxide solution was added until a point was reached at which the hitherto suspended colloidal precipitate of barium phosphotungstate coagulated suddenly and settled quickly to the bottom of the beaker. The heavy sediment was quickly removed and the slight excess of barium was precipitated with sulphuric acid.

A 30 per cent solution of mercuric chloride in alcohol was now freely added in small portions at a time. A small quantity of a flocculent precipitate settled out on standing and was removed by filtration. To the filtrate sodium hydroxide solution was added drop by drop with constant stirring, when a whitish flocculent compound fell out and settled rapidly to the bottom of the flask. This precipitate ceases to appear when the neutral point is reached—or, more correctly, at the first indication of alkalinity—provided that an excess of mercuric chloride is present. The precipitate was collected and washed by grinding it up repeatedly in a mortar with warm water containing a little mercuric chloride until it was free from sulphates.

The mercuric precipitate was next ground up in a mortar with 0.5 per cent nitric acid. With the exception of an inconsiderable flocculent residue, the precipitate dissolved with great ease in this strength of acid. We find that this behavior is characteristic of the mercury compound obtained with a secondary albumose which we have prepared by digesting pig's thyroid glands with pepsin and hydrochloric acid.

The mercury may now be removed with hydrogen sulphide, although this is not always easy as the precipitate may remain in colloidal suspension. Warming on the water bath and the addition of a little nitric acid will generally induce coagulation of the mercuric sulphide. The removal of the mercury is not essential, however, as for the next step, which consists in the preparation of a silver compound, may be used either (*a*) the solution which has been freed of mecury with hydrogen sulphide, or (*b*) the above mentioned 0.5 per cent nitric acid solution of the mercury compound. An excess of silver nitrate is added, and in case solution *b* has been used the precipitate of silver chloride is allowed to settle out. From the filtrate the white flocculent silver compound is precipitated out by the addition of dilute sodium hydroxide. This can be done even in the presence of mercury (solution *b*), since the silver compound falls out while the solution is still markedly acid. It is collected at the pump and thoroughly washed. Considerable loss of material may be sustained here, as water dissolves much of the silver proteose compound after the greater part of the electrolytes has been washed out of the precipitate.

The silver compound is decomposed in a mortar with a small volume of hydro-

chloric acid in excess and the clear filtrate is dropped into several volumes of absolute alcohol, precipitation being completed by the addition of ether. The "hydrochloride" thus thrown out is collected and washed with a mixture of absolute alcohol and ether (1:1).

In case the silver compound has been made from solution *b* (mercury still present) the final product obtained as just described will still contain a small amount of mercury which, however, is readily removable with hydrogen sulphide.

The dried hydrochloride is next dissolved in water and freed from hydrochloric acid by agitation with silver carbonate, and in this way the free albumose is obtained. At this point we may have in hand a preparation which is highly active for the virgin uterus of the guinea pig, giving an almost maximal contraction in a strength of 1:1,000,000. This is true more especially in case the phosphotungstic precipitate above described has not been subjected to reprecipitation and thorough washing. Secretine may also be present in such preparations. The ninhydrin reaction is also given promptly when a solution of the albumose is boiled for a few minutes with the reagent. The contaminating substances are removed by repeated solution in hot water and precipitation from hot absolute alcohol; also by forming a picrate, dissolving it in 20 per cent alcohol containing sulphuric acid and precipitating with absolute alcohol and ether. The free picric acid is washed away from the precipitated albumose sulphate with absolute alcohol and ether, and the albumose will now generally be found to be devoid of physiological activity. If this is not the case some of the above operations, especially the use of picric acid, should be repeated.

As thus obtained the substance has all the characteristics of a pure secondary albumose:

1. It is freely salted out when its solutions are saturated with ammonium sulphate.

2. It gives the biuret reaction, 1 mgm. to the cubic centimeter, with a fine reddish purple color. With large volumes the reaction is still good when the substance is used in the proportion of 1:10,000.

3. The Pauly reaction is immediately positive.

4. Millon's reaction is negative (with 5 mgm. to the cubic centimeter). Only thoroughly purified preparations give a negative Millon. Our notes call attention to the fact that an albumose "sulphate" which was made from a picrate may still give a positive Millon and only after being treated once more with mercuric chloride and silver nitrate will the albumose give a negative response to this reagent. We are no doubt dealing here with a number of secondary albumoses of variable composition.

5. Knoop's reaction is negative (10 to 20 mgm. to the cubic centimeter). Bromine water is immediately decolorized in the performance of this reaction and a white granular bromine compound is thrown out in the presence of a little sulphuric acid.

6. Weidel's reaction is negative.

7. Addition of potassium ferrocyanide and acetic acid to a solution of the albumose causes no precipitation whatever.

8. Tannic acid gives a flocculent precipitate only when a little mineral acid is added.

9. The substance is laevo-gyrous. The specific rotation of the sulphate was found to vary from $[\alpha]_D = -72.2°$ to $-94.4°$, according to the method of preparation.

10. The α-amino nitrogen content of a specimen of the hydrochloride, which in neutral solution failed to give the ninhydrin test (10 mgm. in the cubic centimeter), was found to be 0.53 per cent as estimated with the Van Slyke apparatus. After hydrolysis with 25 per cent hydrochloric acid under a reflux condenser until the biuret reaction had disappeared the α-amino nitrogen was increased about five times. Unfortunately the data for the hydrolysis experiments were lost and therefore exact figures cannot be given.

11. The ninhydrin reaction is negative with the purer preparations when neutral solutions containing 10 mgm. to the cubic centimeter are boiled in the usual way with the reagent. The reaction is, however, always positive when the reagent and the albumose solution are evaporated together to dryness on the water bath and the dry residue is taken up in very weak alcohol or in water (3).

12. Solutions of the albumose may be boiled with 25 per cent hydrochloric acid or with strong nitric or sulphuric acid without developing a dark color or depositing humus substances.

ALBUMOSES IN THE GASTRIC MUCOSA

We have found that albumoses may also readily be prepared from the well washed mucous membrane of the pig's or dog's stomach (fed animals) when the organ is worked up immediately after its removal from the body. Care was taken to keep the mucous scrapings neutral to litmus during the half hour's boiling with 50 per cent alcohol, and under these circumstances an albumose could hardly have been formed as an artefact. When the process is carried through the lead subacetate stage, with removal of the lead and precipitation with alcohol, a white

powder is obtainable which contains albumoses, shows marked activity for the intestinal and uterine strip and has also some blood pressure raising power.

RELATIVE AMOUNTS OF ALBUMOSE IN THE GASTRIC AND INTESTINAL MUCOSA OF A DOG IN DIGESTION AND OF A STARVING DOG

The following comparative experiments were made under as nearly identical conditions as possible on two dogs of approximately the same weight, one of which had been fed with meat six and a half hours before being killed while the other had had nothing but water for four days.

The dogs were bled to death, the stomach and small intestine (as far as the caecum) immediately taken out, slit open, cut into pieces of convenient length and washed thoroughly, first under the tap and then in physiological salt solution, and pressed upon absorbent paper, the surface of the mucosa being gently wiped with paper.[5]

The mucous membrane of the stomach and gut was next scraped off on a glass plate and the weighed scrapings were slowly treated, with vigorous shaking, first with 50 cc. of 50 per cent alcohol and then with 95 per cent alcohol, 1 cc. of the latter being used for each gram of the scrapings.

To the resulting only slightly acid suspension was then added 6 drops of saturated sodium carbonate solution and the mixture was boiled half an hour under a reflux condenser. The supernatant liquid was poured off through a filter paper on a Büchner funnel and the coagulum boiled out three times with 125 cc. of 50 per cent alcohol, then pressed as nearly dry as possible on the funnel and finally dried to constant weight in an air bath.

The combined turbid aqueous alcoholic extracts, after removal of 10 cc. for a determination of the amount of dissolved matter, were next treated successively with neutral lead acetate, basic lead acetate and sulphuric acid, care being taken not to use too large an excess of the reagents, especially in the removal of the lead with the sulphuric acid. In each case the precipitate was first filtered off with the aid of the pump and sucked as dry as possible, and the filtrate, which was always slightly turbid, was repeatedly passed through a folded filter until clear.

The final filtrates were concentrated to a small volume on the water bath under a fan, filtered and treated, with vigorous shaking, with 10 vol-

[5] In the washing, the adherent bile, mucus and products of digestion were removed by rubbing the mucosa gently with the fingers.

umes of absolute alcohol. The resulting flocculent precipitates were collected, after standing some time, as completely as possible on weighed papers, washed with 10 cc. of 10:1 alcohol, dried *in vacuo* over sulphuric acid and weighed.

The two alcohol precipitates were now dissolved in a known volume of water and sodium hydroxide and copper sulphate were added, when it was observed that the biuret color was many shades deeper in the solution derived from the fed dog than in that obtained from the starving dog. Colorimetric estimation showed that if the amount of biuret-yielding substance in the precipitate from the starving dog be taken as 1, that obtained from the fed dog must be rated as 7. Using a secondary albumose in our possession as a colorimetric standard, we found that the actual amount of albumose recovered by us from the gastro-intestinal mucosa of the starving dog was 8.2 mgm. while the amount recovered from the mucosa of the fed dog was 57.4 mgm. It is evident that whether we base our calculation on the comparative weights of the moist scrapings or on the weights of the total dry matter in them (see the table below), there is no escape from the conclusion that much more albumose is present, weight for weight, in the gastro-intestinal mucosa of the fed dog. Taking the gram of dry matter as the unit of calculation, the mucosa of the fed dog is found to contain nearly five times as much albumose as that of the starved dog.

The data from these two experiments are given in the following table.

	FED DOG	STARVED DOG
Weight of dog	16.5 kgm.*	15.5 kgm.
Weight of moist scrapings	326.5 gm.	225.5 gm.
Weight of dry matter in coagulum	47.8 gm.	32.7 gm.
Weight of dry matter in extract	13.1 gm.	8.6 gm.
Weight of total dry matter in scrapings	60.9 gm.	41.3 gm.
Final volume of concentrated filtrate	25.0 cc.	20.0 cc.
Weight of alcohol precipitate	415.0 mgm.	266.0 mgm.
Weight of total albumose in precipitate	57.4 mgm.	8.2 mgm.
Weight of albumose per gram of moist scrapings	0.176 mgm.	0.036 mgm.
Weight of albumose per gram of dry matter in scrapings	0.942 mgm.	0.199 mgm.

*Approximately. The dog was weighed after death and allowance made for the stomach, intestine and blood.

An earlier experiment with two large dogs of approximately equal weight, in which, however, no account was taken of the weight of dry

matter in the coagulum and in the extracts, gave results analogous to the above although quantitatively different. In this earlier experiment the mucosa of the fed dog was found to contain about three times as much albumose as that of the starved dog.

That we do not in these experiments carry our processes through to the end and prepare the albumoses in a pure state cannot invalidate our conclusion that the gastro-intestinal mucosa of a fed dog contains much more albumose than that of a starved dog. At the stage of our process at which the precipitates were compared, protein matter is present in the form of albumose only. Albumose which responds to the Millon reaction is still present, though later this variety may be lost in the various chemical steps that follow the use of lead subacetate.

ALBUMOSES OF THE PIG'S THYROID GLAND

In order to obviate the criticism that the albumoses here described are products of autolysis we proceeded as follows in working with this gland. By special arrangement with the manager of the slaughter house we were enabled to receive the glands still warm from the hand of the butcher. The glands were cut into a few pieces and dropped at once into boiling 50 per cent alcohol. In this way approximately 1 kgm. of fresh thyroids was boiled up at the slaughter house, it being our purpose to destroy the ferments of the gland. The alcoholic solution was removed, the cut up glands were ground up in a machine and again extracted with hot 50 per cent alcohol. The alcoholic extracts were now treated in the manner described for the intestinal mucosa, up to and through the lead subacetate stage, including the removal of the lead as lead sulphate. The lead-free solution was concentrated under the electric fan at a low temperature and was then dropped into absolute alcohol and the precipitate collected and dried. This precipitate, a water-soluble white powder, gave no turbidity with potassium ferrocyanide and acetic acid, and was therefore quite free from coagulable proteid. The product was now carried through the mercuric chloride and silver nitrate treatments. It is to be noted again that the solution of the mercuric chloride precipitate in very dilute nitric acid (0.5 per cent) excludes the presence of coagulable proteid, even if the earlier steps had not already insured us against this. The silver compound was made from the nitric acid solution without first removing the mercury. From the silver compound the hydrochloride of the albumose was prepared as a dry powder in the manner already described.

This product gives the biuret reaction in the manner characteristic of albumoses, while the Millon reagent with 20 mgm. to the cubic centimeter did not give a fine red color or a red precipitate but only a yellowish tinge. In other respects the product behaved like the albumose obtained from the intestinal mucosa. The quantity of this albumose obtainable from the thyroid gland is small as compared with that found in the intestinal mucosa. We judge that we had about 0.1 gram of dry product after having completed the lead subacetate step in our method, a quantity equivalent to 0.01 per cent of the weight of fresh bloodless glands. The amount of pure albumose present in the gland is probably lower than this figure.

ALBUMOSES IN STRIATED MUSCLE

In order that the conditions of the extraction might be entirely under our control we made use of the skeletal muscle of the dog. The animals used had not been fed for sixteen hours. They were etherized and bled to death from the carotid artery, whereupon some of the larger muscles were rapidly stripped from the limbs and run through a meat grinder. From one animal 760 grams of ground up muscle were obtained in this way, from a second 784 grams. Each lot was worked up separately and was carried through the lead subacetate stage of our process. The lead-free solution was precipitated from an excess of absolute alcohol in the presence of a slight excess of sulphuric acid. The albumose fell out as a white flocculent precipitate, contaminated, as usual at this stage, with other constituents of the tissue, notably amino acids. It seemed hardly necessary to apply further processes of purification as it was evident that this precipitate was free of coagulable proteids. Approximately 0.01 gram dissolved in a few drops of water gave only the merest trace of precipitate with potassium ferrocyanide and acetic acid on long standing. So, too, the addition of uranyl acetate to a solution of the albumose sulphate caused the appearance of only a faint turbidity which finally aggregated to a small amount of a flocculent precipitate. The filtrate from this precipitate, after removal of the uranium with sodium hydroxide, gave the biuret reaction with great intensity.

Our experiments show that under the conditions here described only a small amount of albumose exists in dog's muscle, and it is doubful if much more is obtainable from this tissue even when the animal is in full digestion.

Skeletal muscle tissue (920 grams) of a third dog, also unfed for sixteen hours, was first boiled for half an hour with distilled water, then pressed to a hard cake and the filtrate discarded. The disintegrated cake was then boiled up with an equal weight of 50 per cent alcohol and the alcoholic filtrate was treated in the manner described for the intestinal mucosa up to the stage at which lead acetate is used. The powder obtained at this point (which is ordinarily the starting point for the treatment with lead acetate) failed to give a biuret reaction and it therefore was unnecessary to proceed further with this control experiment. It shows that the albumose present in the muscle was completely removed by the boiling water.

The methods heretofore applied to this tissue have failed to detect the very small amount of albumose which we have been able to extract from it with 50 per cent alcohol. Thus Whitfield (4) failed to find a proteose in rabbit's muscle, either when he treated a 0.65 per cent sodium chloride extract with trichloracetic acid or with saturated ammonium sulphate solution. No data are given as to the quantity of tissue extract used in the separate tests. The biuret reaction is not a sensitive test for albumoses—the reaction is not obtainable with a secondary albumose in a concentration below 1: 10,000 or 1: 12,000. We have usually made this reaction with 0.1 per cent solutions of albumose, that is, in a concentration of 1: 1,000. Then, too, a number of disturbing factors must be taken into account in applying this test. At the concentration of albumose of 1: 2,000 an excess of dextrose, if present, or a yellow color in the solution will entirely cover up the biuret reaction, and a negative result under these conditions is not conclusive. It is therefore advisable to make the test with an isolated precipitate rather than with a complex solution.

v. Fürth (5) and Mays (6) also report that they could not detect a true albumose in muscle by the methods employed by them. Mays, however, was able to separate, by salting out with magnesium sulphate, considerable quantities (140 grams from 1814 grams of extract) of a proteose from a Liebig extract prepared in South America. The substance was not put through a series of purifications as in our work above, and Mays is undecided whether to call it an albumose or a gelatose. He thinks it very unlikely that it is an albumose which has been formed by the action of hot water (84° to 94°) on the chopped up meat and he falls back on the supposition that it is most likely a gelatose, although admitting the difficulties that stand in the way of this suggestion. In the first place, the chemical reactions cited by Mays do not speak

conclusively for gelatose, and the processes employed in manufacturing the extract can lead, as Mays admits, to the conversion of glutin into gelatose only if a number of quite unproved assumptions are made in regard to the favoring actions of salts and other constituents of the extract in facilitating this conversion.

Under the circumstances and in view of our findings, we prefer to believe that the albumose of meat extracts is accounted for by the presence of this substance in the muscle tissue and that it has been concentrated in the extract by the processes of manufacture, as is the case, for example, in the pituitary extracts of commerce. Mays found that meat extract contains 7.8 per cent of his albumose. Pituitary extracts, as we have shown in an earlier paper (7), may contain fully 10 per cent of albumose.

ALBUMOSES IN THE GRAVID UTERUS

The uterus was removed by Caesarean section from a woman at full term by Prof. J. W. Williams and was brought to the laboratory after having lain ten hours on ice. The decidua and mucosa were scraped from the fundus and the organ was then ground up and boiled with alcohol in the usual manner. The processes were carried through the lead subacetate stage and precipitation with alcohol gave a white powder which gave a colorless solution in water and which responded to the tests for albumose already described. The yield of albumose appeared to us to be greater than in the case of skeletal muscle.

ALBUMOSES IN OTHER ORGANS

We have not made a systematic search for albumoses in other organs It may be of interest, however, to state that an examination of a soluble extract of the *corpora lutea* which is sold to physicians by one of our large manufacturing firms behaves in respect to the proteid precipitants and reactions described in this paper in such a manner that one can only conclude that proteoses are present in the extract.

Each cubic centimeter of the extract contains the soluble constituents present in 0.2 gram of the dried corpora lutea. The solution gives no precipitate on boiling after the addition of a trace of acetic acid, nor does it give a precipitate with potassium ferrocyanide and acetic acid; the biuret reaction is positive and gives the reddish color characteristic of albumoses; the Pauly reaction is also given immediately. From these reactions one must conclude that we are dealing here with an

extract which is free from coagulable proteids but still contains a proteose in considerable quantity.

A second commercial preparation which we have not ourse'ves examined but which undoubtedly contains an albumose is the "Hormonal" of Zuelzer. This extract is prepared from the spleen of cattle and has been shown to have some value as a motor stimulant for the intestine. In a few instances severe collapse and even death have followed the intravenous injection of 20 cc. of the preparation. It was shown by Dittler and Mohr (8) that Hormonal contains powerful blood pressure lowering substances. The fatalities, as well as the cases of dangerous collapse, are fully accounted for by these depressor substances. We would note in passing that intestinal extracts also contain these very powerful blood pressure lowering substances and that they can be completely removed by the proper use of alcohol and basic lead acetate. Zuelzer (9) believed that the untoward effects observed were caused by the presence of a toxic albumose and took steps to have this substance removed. Dittler and Mohr (10), however, found on examining the new Hormonal that it still contains depressor substances, and the physiological chemist, Professor Siegfried, who also examined the new preparation, declared it to contain albumoses.

We have here, then, an extract of the spleen, which was no doubt prepared from perfectly fresh mat:rial, which contains albumose. That the extract still contains blood pressure lowering substances is due to faulty technique.

ALBUMOSES IN THE BLOOD

A number of investigators, notably Hofmeister and his school, as also E. Freund and his pupils (11), have always maintained that blood plasma and blood serum contain albumoses in small quantity. The opposite opinion has been upheld energetically by Neumeister and Abderhalden and their pupils (12). Howell also was not able to convince himself that blood contains a non-coagulable proteid of a proteose nature. He found (13) that

> Blood serum from the fed or starved animal when subjected to dialysis in colloidon tubes gives no indication of the presence of a perceptible amount of peptone or proteose.

That both the blood and the urine may contain albumoses in pathological states of the organism, in which absorption of broken down cells occurs on a large scale, is generally admitted, but we shall not here consider this aspect of the question.

We have repeated the work of the later investigators of the Hofmeister school, Hohlweg and Meyer, and have found, quite in agreement with their statements, that whole blood, serum or plasma which has been freed from coagulable proteids by their method gives a negative ferrocyanide *and a positive biuret test.* Apparently such fluids should contain a proteose. The biuret-yielding material can be salted out from such solutions by the use of ammonium sulphate and heat, but we have not been able to identify the proteid precipitate thus obtained as a proteose. If originally present as an albumose, it must have been changed into an insoluble dysalbumose in the course of our operations, since the final product obtained after removing ammonium sulphate from the precipitate is no longer soluble in distilled water. Such negative results were obtained even with as much as two liters of blood, both with the original method of Hohlweg and Meyer and with a n.odification of it devised by ourselves which obviates dilution of the plasma.

When we applied our alcohol-lead acetate-subacetate method to the plasma (oxalated or citrated), only negative results were obtained with both portal and systemic blood. One element of uncertainty inheres, however, in this negative finding by this particular method—the final product here obtained always gave a yellow solution and in such a solution it is impossible to determine with certainty, by means of the biuret reaction, whether small quantities of a proteose are present.

Positive results were obtained when this method was applied to the mass of red and white corpuscles that were obtained by centrifugalizing two liters of systemic blood. The final precipitate in alcohol, after removal of lead by the method previously outlined, gave the tests for albumose so unmistakably that it seemed unnecessary to proceed further with the purification of the precipitate. The quantity of albumose obtainable from the cellular elements of the blood by boiling with 50 per cent alcohol is, however, not large but suffices for identification.

DIALYZATES OF PORTAL, HEPATIC AND SYSTEMIC BLOOD PLASMA

Oxalated plasma as obtained by centrifugalizing blood from the portal and the hepatic veins, as also from the carotid artery, was submitted to dialysis during periods varying from three to fifteen hours in series of celloidin sacs[6] and tubes. The fluid on the outside of the sacs was dis-

[6] The celloidin solution here used was like that described in the Journ. Pharm. and Exper. Therap., 1914, v, 279, also ibid., p. 611. In this work on vividiffusion

tilled water in some experiments; in others it was an 0.8 per cent solution of sodium chloride. In the course of a few hours considerable biuret-yielding material passes through the walls of these celloidin sacs and tubes. At first precautions were taken to collect the blood in sterile fashion and to sterilize both the sacs and the vessels in which the dialysis was carried out, but as it was found that these precautions were of no advantage in experiments of short duration they were discontinued. It is possible to overlook the proteid material that passes through celloidin sacs if the biuret test is applied to the concentrated dialyzate without first removing the sugar with boiling 93 per cent alcohol. In this way dextrose and other disturbing substances are removed, and a concentrated residue which at first fails to give the biuret reaction will now give this test very beautifully.

Considerable quantities of this diffusible proteid were collected by concentrating the distilled water dialyzates of various kinds of plasma. In each case the attempt was made to isolate a proteose from the partially concentrated fluids by the employment of lead acetate and basic lead acetate for the removal of coagulable proteids but always the attempt ended in failure. We are well aware that certain primary albumoses are precipitated by basic lead acetate and that the absence of a biuret-yielding substance in the filtrate from the lead precipitate does not exclude the presence of this class of proteoses in the dialyzates. But if they are present, they are easily changed into insoluble dysalbumoses. Skins of coagulated albumin are formed during the concentration of the dialyzates on the water bath under the electric fan, and when sufficiently concentrated exhaustion of the residue with 93 per cent alcohol causes it to become entirely insoluble in distilled water. As far as we have gone, then, *we can only say that our methods have not enabled us to separate a true proteose from the proteids that pass freely through celloidin membranes during the first few hours of dialysis.*

The inherent difficulties in the way of separating albumoses from the blood are well illustrated in the following experiment. Corrosive sublimate, as is well known, is an effective agent for removing coagulable proteids from albuminous solutions. We desired to learn if a secondary albumose added to whole blood in known quantity could be recovered

(1914) Abel and his associates also failed to isolate a proteose from their diffusates, in spite of the fact that a secondary albumose (Hirudin) was constantly being run into the veins of the animal used. As in the present experiments a coagulated proteid was always found in small amount in the evaporated dialyzate, which goes to show that the diffusible proteid here described exists preformed in the circulating blood.

when this agent is employed. Accordingly 0.15 gram of a pure secondary albumose dissolved in 7.5 cc. of 0.8 per cent sodium chloride solution was injected directly into the outflowing stream of blood from the portal vein of a dog. The portal blood with the admixed albumose was then whipped with a wire brush and filtered through cotton. The filtrate amounted to 96 cc. and should have contained the added albumose (0.15 gram) except in so far as it was absorbed by the fibrin. The defibrinated blood with its albumose was now treated according to the directions of Gayda (14). It was stirred into 480 cc. of 2 per cent mercuric chloride in 0.8 per cent hydrochloric acid, frequently shaken, let stand overnight, filtered through paper, freed from mercury with hydrogen sulphide and from the excess of the hydrogen sulphide with a current of air, neutralized with sodium hydroxide and concentrated under a fan at about 55° to a volume of 35 cc. The flocks which separated out gave no biuret reaction. The filtrate when further concentrated likewise gave no biuret reaction. So also the precipitate obtained by adding alcohol failed to give even a trace of a biuret reaction. Evidently the added albumose was lost entirely in the course of our manipulations. It must be borne in mind that the secondary albumose here employed is not precipitated by mercuric chloride in the presence of dilute acid when tested by itself in aqueous solution.

On the other hand, when this albumose was added to oxalated plasma in the proportion of 0.005 gram to the cubic centimeter and the plasma was then dialyzed against distilled water it was not difficult to show that a little of the albumose passed into the dialyzate. Ordinarily, when the dialyzate is concentrated nearly to dryness under the electric fan at as low a temperature as possible and then exhausted with hot 93 per cent alcohol the proteids present become insoluble in water, as has already been stated. In the present instance, however, a water-soluble proteid was found in the final residue and this could only be the albumose originally added to the plasma. In conclusion, it should be stated that the amount of albumose recovered in the dialyzate appeared to be much less than we had a right to expect.

OTHER INSTANCES OF THE PRESENCE OF ALBUMOSES IN TISSUES, AS DESCRIBED IN THE LITERATURE OF BIOLOGICAL CHEMISTRY

As already mentioned in this paper, Abel and Pincoffs (7) have shown that all pituitary preparations examined by them contain albumoses.

Slowtzoff (15), in reporting the results of his analyses of human semen, writes as follows:

Human semen contains, in addition to a nucleoproteid, traces of mucin and albumin, an albumose-like substance which in respect to its precipitation limits and its chemical reactions must be regarded as a primary albumose.

Szumowski (16), who made alcoholic extracts of the finely comminuted organs of geese, doves and dogs in a search for zein which had been administered to these animals, states that he found a biuret-yielding substance which was not zein in a number of instances. He is at a loss to explain his findings. We have no doubt that had this author treated his alcoholic extracts in the manner described by us, thus removing sugar and other disturbing substances, a positive biuret reaction would have been obtained by him in many more of his extracts. We have cited this author's findings because we believe that they are explainable only on the basis that he has unwittingly extracted albumoses from a number of tissues.

Wohlgemuth (17), who has made a study of human bone marrow under pathological conditions, precipitating the coagulable proteids by boiling with dilute acetic acid and sodium chloride up to 1 per cent until the filtrate gave no turbidity with potassium ferrocyanide, reports as follows in regard to albumoses in this tissue: Albumoses were present in one case out of five of yellow bone marrow, and out of six of red bone marrow four contained albumoses. The marrow was taken from the femur in each case.

It is not our purpose to sift the voluminous literature pertaining to the appearance of albumoses in the urine in various pathological conditions. In the great majority of instances the excreted albumoses appear to be of the secondary type (18) and there is at present good reason for believing that this is accounted for by the absorption into the blood of the constituents of disintegrating tissues. The frequent appearance of albumoses in the urine under circumstances where tissue breakdown obtains, lends support to the view that the albumoses found by us in various tissues were actually present in them and were dissolved out of them by the boiling 50 per cent alcohol used in our experiments.

Panzer (19) has isolated albumoses from the sporozoites of a species of Coccidia known at *Goussia gadi*.

DISCUSSION

It has been shown that albumoses may be isolated from the tissues by extracting them with boiling 50 per cent alcohol and removing coagulable proteids from the alcoholic extract. In the case of the intestine

and the thyroid gland the isolation was carried through so many processes that the secondary albumose finally obtained was as nearly a chemical individual or entity as present methods permit us to isolate, and even in the case of other tissues the isolation had proceeded far enough to dispel all doubt that we had an albumose in our hands. Where, as in the single instance of the plasma of the blood, the isolation could not be effected, we have so stated and have not accepted the positive qualitative tests of earlier investigators as proof of the existence of albumoses in this fluid.

But the actual isolation of an albumose from a tissue is in itself no proof that it was present as such in the cells of that tissue. The albumose may have been formed from the proteids of the tissue in the course of the chemical manipulations employed for their isolation. We have given special attention to this phase of the question and offer the following considerations in support of our belief that albumoses actually exist in the cells of the body, being stages in the metabolism comparable with the amino acids and other intermediary products. We employed boiling 50 per cent alcohol because it is a good solvent for our albumose and because it thoroughly disintegrates the cells of the tissues and coagulates their proteids. The time of boiling was originally set at one hour in the case of the intestinal mucosa because we desired at that time to extract completely other substances than albumose. As stated elsewhere, we have frequently extracted tissues for half an hour and we have no doubt that where it is desired to obtain only the proteoses boiling the finely divided tissue for a quarter of an hour will amply meet the requirements of the case. Weak alcohol is an indifferent fluid and can not of itself hydrolyze proteids to albumoses within the above time limits, if at all.

It may be argued that the acidity of the dead tissues was sufficient to cause the formation of small quantities of albumoses in the boiling extracts. We think that this also can not be urged as the source of the albumoses. In a number of instances, as in experiments with the gastric and intestinal mucosa of fed and starved dogs, great care was taken to boil at the neutral point to litmus and not to concentrate the alcoholic extracts until after the use of lead acetate and basic lead acetate and after the removal of lead with sulphuric acid. These precipitations were all made at room temperature and the final filtrate no longer contained a coagulable proteid at the time the alcohol and acetic acid were driven off. Even when this final alcoholic filtrate was concentrated under reduced pressure, after having neutralized the acid,

the results remained the same. Finally, it is difficult to see (on the supposition that our albumose is an artefact) how so much more albumose can be obtained from the gastro-intestinal mucosa of the fed dogs than from that of starved dogs. It will be noted that the amounts of albumose which can be isolated in the two cases stand in no relation to the amounts of proteid (total dry matter) present in the two mucosae.

Post mortem autolytic changes, while possibly responsible for a part of the albumose present in the pituitary and other extracts used in medicine, certainly play no rôle as a causative factor in the experiments described by us, in which tissues were taken directly from animals just killed. Autolysis can not therefore be held responsible for the proteoses isolated by us.

Our observation that the gastro-intestinal mucosa of fed dogs contains much more albumose than is found in the corresponding mucosa of starved dogs calls for a word of discussion. In this connection the question naturally arises whether it is possible to wash away from the mucosa all adherent proteose, retaining only that part that has entered the cells of the mucosa. We answer in the affirmative, if the washing is done under a jet of running water and the surface of the mucous membrane is stretched[7] and gently rubbed with the finger, if the pieces of intestine are then soaked and again rubbed in large bowls containing 0.8 per cent sodium chloride solution and if, finally, the surface of the intestine is pressed down on filter paper and gently wiped with pieces of this paper. All this was done with the stomach and intestines of the dogs used in our experiments.

We also find support for our opinion that a mucous surface can be washed free from adherent proteid material in the extensive literature of physiology in which quantitative experiments are described in which loops of intestines or gastric pouches are treated as receptacles, the assumption being made that these living receptacles can be freed of their contents and washed, much as is done with laboratory utensils.

But we can offer a more incontrovertible proof of the truth of the statement that freely soluble substances, such as peptones and albumoses, can be washed away completely from the surface of the intestines, by citing our earlier experiments with certain phthaleins (20).

In these experiments 20 to 30 cc. of a 2 per cent solution of tetrachlorphthalein were injected into dogs subcutaneously. After the ex-

[7] It is especially necessary to stretch the walls of the stomach while washing so that the folds of the mucosa may present a level surface to the impinging jet of water.

piration of from eighteen to thirty hours the animals were killed, the large and the small intestines were removed separately, opened and washed perfectly clean under running water. Before washing, the application of a 5 or 10 per cent solution of sodium hydroxide to the mucous membrane of the small intestine always gave an intense deep red stain over large areas. This was especially intense where mucus was abundant. On applying the alkali to the *well washed* small intestine, however, *no color reaction could be obtained.*

It only remains to add that the mucosa of the large intestine could not be washed free of the phthalein but always took on an intense, brilliant deep red stain throughout its whole extent upon the addition of alkali. Frozen sections showed that the drug was contained not only in the cells of the mucosa but was present also in the submucosa. Similar sections of the small intestine were found to be free of phthalein. Further research proved that the phthalein was excreted by the liver and was carried down the intestine in a state of solution and reabsorbed by the large intestine only, the absorption beginning abruptly at the line where the small intestine merges into the large.

When the delicacy of this color reaction is borne in mind, when we consider that the mucous surface of the small intestine, which has been bathed throughout its entire length with a solution of the phthalein, could be washed entirely free of the compound while no amount of washing could remove it from the mucosa of the large gut (the compound having entered the cells of this organ), we cannot escape the conviction that soluble and merely adherent compounds are completely removable from the mucous surfaces of the intestines or stomach.

In the light of the above experience we must conclude that that portion of proteose (as in the case of the phthalein in the large gut) which could not be washed away from the gastro-intestinal mucosa had actually been taken up by the absorbing cells.

At the present writing we can not trace the absorbed proteoses beyond the mucous membrane and, strictly speaking, it is only an inference on our part that they are absorbed as such and not produced by synthesis from absorbed amino acids. Nolf (21) and Asher (22) have shown that the intestines can take up proteoses, and we know that these derivatives of proteids are always present in the digestive canal during digestion. It seems far fetched, therefore, to assume that the increased amount of proteose found by us in the gastro-intestinal mucosa of fed dogs was not absorbed as such but was made by synthesis. Certainly, the assumption that these soluble proteoses are taken up by

the cells from the digested matter with which they are in contact seems the more rational point of view.

There is much controversy in this field. Abderhalden (23), in a review of the subject in the last edition of his text book, after weighing the facts pro and con, leaves it undecided whether higher peptids are absorbed by the intestinal mucosa and does not commit himself unreservedly to the theory that amino acids only are absorbed. E. Zunz (24) shows that proteoses disappear both from the stomach and the intestines but concludes that one can not decide how much of the absorbed nitrogen is taken up in the form of higher split products, how much in the form of lower, because of the reversible action of ferments, and so leaves the matter in an unsettled state. London (25) and his collaborators maintain that no absorption takes place from the stomach during digestion, while Tobler (26), Lang (27), Salaskin (28), Scheunert (29) and Grimmer (30) all hold that the products of the gastric digestion of proteids (proteoses) are absorbed in the stomach, merely differing among themselves as to the quantitative factor in the absorption.

The amount of proteoses or higher polypeptids taken up by the gastric and intestinal mucosa during a digestive period must be very considerable, as judged by our findings. In the case of the amino acids it has been shown that the mesenteric blood may contain twice as much amino acids after feeding with meat as before feeding (31). But in the case of our albumoses we are halted at the point of intake and cannot follow them in their passage into the plasma. As we have seen, we were not able to isolate a proteose from plasma. For the present, therefore, we can only speculate as to the fate of the proteoses that have entered the cells of the gastro-intestinal mucosa. Are our methods for separating them from the proteids of portal plasma at fault? Are they split up into abiuret products in the mucosa and do they pass into the blood stream only in the form of such abiuret products? Are they built up in the mucosa into *less diffusible proteids which have lost most of their proteose characteristics?* The knowledge at hand permits of no conclusive answer to these questions. Nevertheless, it must be regarded as a step forward that so marked an increase in the proteose content of the gastro-intestinal mucosa can be shown to take place during digestion. This observation (if it be granted that these proteoses are actually absorbed and not produced by synthesis) certainly militates against the theory now so widely advocated that proteids are broken down completely into amino acids in the digestive canal and absorbed only in this form.

SUMMARY

1. Albumoses can be isolated in varying amounts from the tissues of the body, inclusive of the cellular elements of the blood. The methods employed did not, however, enable us to separate a proteose of any kind from the plasma of the blood.

2. To prepare an albumose from the gastric or intestinal mucosa which is entirely devoid of pharmacological activity (pressor, oxytocic and secretory) requires the employment of numerous chemical procedures, as outlined in this paper.

3. The gastro-intestinal mucosa can be washed free of soluble adherent substances, such as the proteoses. We are justified in assuming that what can not be washed away must be truly a part of the mucosa. The gastro-intestinal mucosa contains from three to five times as much albumose during digestion of meat as after deprivation of all food (except water) for four days.

4. In view of this finding we can not accept the theory that proteids are taken up by the absorbing surfaces of the digestive apparatus only in the form of amino acids, but must believe rather that proteoses as well as amino acids are freely absorbed, as has long been maintained for the former by certain investigators. While we are able to trace the further passage of amino acids from the mucosa to various organs via the blood current, we find it impossible at present to do this with proteoses. These can not be traced further than into the absorbing mucosa, unless it should be assumed that the cellular elements of the blood are distributing agents for them—a point of view which we are at the moment not justified in advancing. The details of the fate of the absorbed proteoses still remain to be determined.

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THE EFFECT OF TEMPERATURE ON THE RHYTHM OF EXCISED SEGMENTS FROM DIFFERENT PARTS OF THE INTESTINE

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It has been shown by Magnus (1) that excised segments of the intestine will beat rhythmically when immersed in Ringer-Locke's solution. He found that contractions would take place between the temperatures of 14° and 49°C., and that the rate and amplitude increase with a rise in temperature up to 42°, when a decrease appears. All movements cease at 47°C. (in the rabbit). In figure 16 of his article, he shows that the curve charted on coördinate paper rises more acutely at lower temperatures and flattens at higher temperatures. He compares his results with those obtained on warming heart muscle. He found temperature changes very constant, "und zwar verhalten sich die verschiedenen untersuchten Exemplare (Katzendarm) durchaus gleichmässig." He probably refers to segments from different cats because if he had taken a number of segments from different parts of the intestine of the same animal, he would probably have noticed the difference in rhythm later pointed out by Alvarez (2).

Alvarez has shown that the rhythmicity rate of different segments from the intestine decreases progressively from pylorus to colon. At 38° those from the upper duodenum beat about 16 times per minute, those from the lower ileum about 10 times per minute. Since recent work indicates more and more strongly that this rhythmic activity arises in the muscle itself and not in the nerve plexus, we must look for differences in the rate of metabolism in the different regions of the gut (3). If we can take a segment of ileum beating at 10 per minute and, by warming it, speed up its metabolism so that it beats at 15 per minute, it seems probable that a segment of duodenum which normally beats at 15 per minute has a faster inherent metabolism than that of

the ileal segment. It is common knowledge that biological chemists who, on account of the complex and delicate nature of living protoplasm, are seldom able to employ the usual analytical methods, oftentimes can learn a great deal about particular living reactions by observing the way in which they are influenced by changing conditions. These processes may thus be found to follow well-known laws that govern simpler and better understood reactions. Thus, by finding the coefficient of acceleration for a degree of rise in temperature, and by plotting curves, they can distinguish between reactions following chemical laws and others following physical laws. The present work was undertaken with the hope that similar studies, in which the effect of a rise in temperature could be observed in the rhythmicity of segments from different parts of the bowel, might throw some light upon their metabolism.

Segments of intestine were cut from (1) the first portion of the duodenum, (2) from the upper jejunum, (3) from the region midway between the duodeno-jejunal junction and the ileo-cecal sphincter, (4) from the lower ileum, and (5) from the colon. The figures obtained from the colonic segments have been discarded as that part of the gut proved to be too erratic in its activities. Healthy rabbits were used. The animals were killed by a blow on the head; segments of intestine were cut immediately and kept in cooled Locke's solution until needed. The five different segments, each about 2.5 cm. in length, were attached to light heart levers and lowered into a beaker containing 400 cc. of aerated Locke's solution at 15°C. The beaker was placed in a water-bath the temperature of which could be raised at will. The contractions of the different segments were recorded on one drum, together with the temperature readings from time to time. A sample tracing is shown in figure 1. Ordinarily the duodenal and ileal segments were the first to show activity. The duodenal and jejunal segments in all experiments showed a definite rise in tone for the first ten to fifteen minutes. Such a rise cannot be attributed entirely to the rise in temperature as it appears in segments beginning and remaining at 38°. The tone generally reached its highest point first in the duodenum. A slight rise in tone was observed on a few occasions in the middle segment. The rise in the colon was less constant and slower in onset.

Seventeen experiments have been done on segments from nine rabbits. The rates per minute have been counted, when possible, for each degree of rise. The results have been so uniform that almost any single experiment would have served to bring out the points to be made

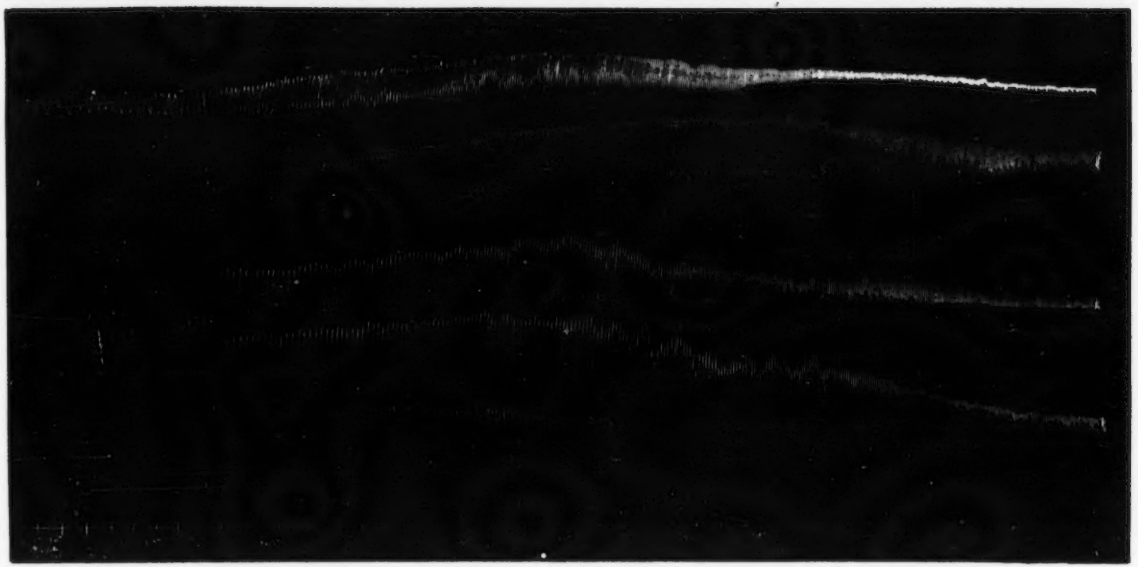

Fig. 1. From above downward the tracings are from segments of duodenum, jejunum, middle of small intestine, lower ileum and colon. Time markings equal 30 seconds. Temperature varied from 19° to 39.5°C.

later. The data from all the experiments have been assembled in table 1.

Averages have been obtained for the rates of the four different segments at the different temperatures, and the logarithms of these aver-

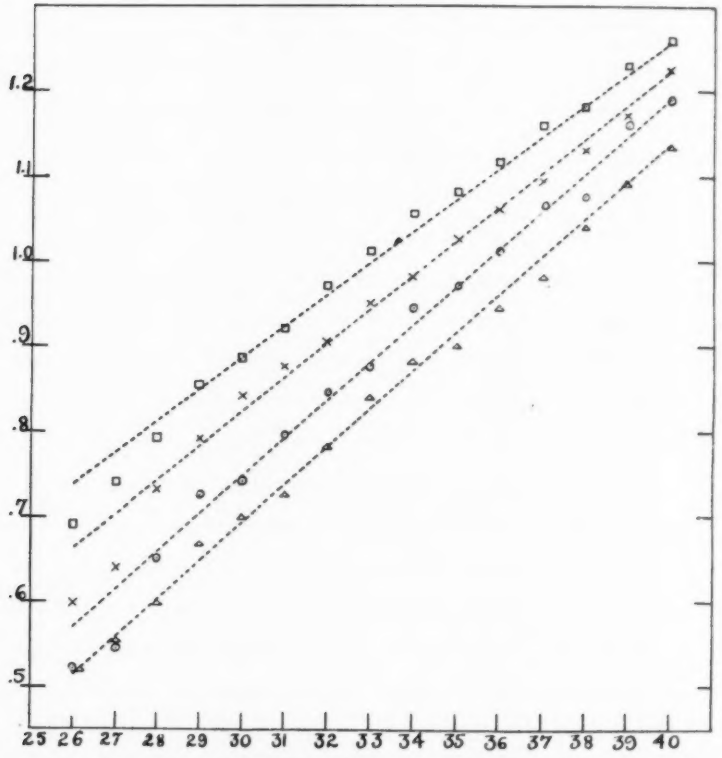

Fig. 2. Ordinates represent logarithms of rate; abscissae represent degrees Centigrade. From above downward the lines are drawn through data from the duodenum, jejunum, middle of small intestine and lower ileum.

ages have been used as ordinates in figure 2. The abscissae represent temperature in degrees Centigrade from 26 to 40. It will be seen that when plotted in this way the data fall in four fairly straight lines which converge toward their upper limits. It will also be noted that at any

TABLE 1

RABBIT	SEGMENT	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°	36°	37°	38°	39°	40°
I	D											8.2		9.4		12	11.1	12		15.2	16.5	17.5
	J											7.0		9.9		11.2	11.0	13		14.0		
	M						3.0			4.0		6.0		7.5		11.0	10.0	12.8		14.0	14.0	14.0
	I						1.1			4.0		5.0		6.2		8.9	9.0	10.5		12.7	14.0	14.8
II	D	2.7		3.4			4.0		5.2	5.8		7.0	7.9	8.0	9.0	10.0	10.2	12.0		14.4		17.0
	J						4.5		5.3	6.0		7.5	7.9	8.0	8.9	10.0	11.0	12.2		14.0		17.6
	M			2.4			3.2		3.6	3.5		5.1	5.6	6.8	7.0	8.5	8.8	8.8		10.5		14.8
	I			2.4			3.0		4.2	4.0		4.5	6.0	6.5	8.0	9.0		13.0		13.5		15.0
III, Set 1	D			2.6			4.2		5.0	5.0		6.0	6.2	8.0	9.0	9.5	11.6	10.7		14.7		18.0
	J								3.5				5.3	6.0	6.3	8.0	9.1	9.5		12.5		
	M											3.6	3.6	4.5	4.0	7.0	7.0	10.5		10.5		
	I								3.5	3.6		5.0	5.0	6.2	6.3	6.6	7.0	7.0		11.0		
III, Set 2	D			2.5								7.0	6.4	8.0	8.3	10.0	10.6	9.0		11.0		
	J											4.4	5.5	6.0	6.1	8.0	8.5	7.5		10.6		
	M											3.4	3.5	5.0	5.0	6.0	7.1	6.7		10.0		
	I											3.5	3.0	4.0	4.5	6.8	7.2	6.5		9.0		
IV	D																					
	J						4.0		4.2	4.8		6.5	7.0	7.9	8.6	9.0	9.2	9.5		11.5		
	M						3.0		2.8	3.4		4.8	5.2	6.0	6.6	7.0	7.5	7.5		9.5		
	I						2.5		3.1	4.0		4.0	4.3	4.9	5.8	6.6	5.5	6.8		8.0		
V, Set 1	D									6.0	6.0	7.2	8.0	8.8	10.0	11.1	11.2	11.0		12.5		17.1
	J									5.0	5.3	6.7	8.5	8.0	9.7		8.9	9.6		12.0		15.0
	M										4.9	7.0	7.5	8.0	8.0	9.5	9.8	10.1		12.0		15.8
	I									3.5	3.8	4.7	5.2	5.9	7.5	7.5	7.9	9.5		9.1		13.1

V, Set 2	D						3.6			4.8		6.0	8.1		11.0	13.2	12.5	12.1		15.5		
	J						3.5			4.8		6.6	9.2		11.7	14.0	11.6	11.2		14.0		
	M												8.1		11.0	13.1	11.7	9.5		12.0		
	I						3.2			3.8		5.1	5.0		8.8	10.1	9.0	9.5		11.5		
VI, Set 1	D									6.5	7.1	7.6	8.5	9.0	10.1	11.9	13.8	14.0	15.3	18.0	18.0	21.1
	J								4.0	5.1	6.0	6.0	7.5	8.9	9.0	10.5	11.8	12.1	13.1	15.0	15.0	17.0
	M									4.8	6.0	5.6	7.1	7.9	8.9	10.0	11.4	11.4	12.6	14.0	14.0	16.5
	I								3.5	4.1	5.0	4.7	5.6	5.4	6.1	6.5	8.3	8.0	8.6	10.0	10.5	12.1
VI, Set 2	D							5.6	8.5	11.3	11.0											
	J							4.9	6.0	7.0	7.9	8.8	9.1	9.1		11.0	12.0	12.1	13.0	14.5	15.0	17.5
	M									5.9	7.0	7.9	8.1	9.8		10.5	11.9	12.9	13.0	14.1	15.0	17.1
	I							2.8	3.0	3.6	4.0	4.9	5.0	6.5		7.5	8.5	9.0	9.4	10.0	11.0	13.0
VI, Set 3	D						6.0			7.9	9.0	10.0	11.0	11.3	11.9	13.3	13.0	14.1	13.0	14.5	18.6	
	J									9.1				11.0	12.1	14.0	12.1	13.2	13.1	14.1	16.0	
	M									7.0		8.3	9.9	9.9	11.0	12.6	11.3	13.0	11.9	13.8	15.9	
	I						2.0			3.9	4.7	5.2	6.0	8.0	8.5	10.1	10.0	11.2	11.9	12.5	15.1	
VII	D									8.0		9.5								15.0		
	J						4.2			6.2		8.0								13.5		
	M						2.8			4.5		5.2								10.7		
	I						3.1			4.5		5.5								11.5		
VIII, Set 1	D											8.0	9.0	8.9	9.5	10.6	11.8	13.0	13.0	15.0		
	J									4.2	5.2	5.9	6.8	7.1	8.0	9.5	10.9	12.1	13.0	14.5		
	M											5.1	5.5	5.6	6.2	7.1	9.0	10.0	11.4	13.5		
	I									3.7	4.7	4.7	5.1	6.0	6.0	6.6	6.4	7.0	8.3	11.0		

TABLE 1—Continued

RABBIT	SEGMENT	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°	36°	37°	38°	39°	40°
VIII, Set 2	D			3.2		3.7		5.0	5.5	6.1	6.7	7.1	8.0	9.0	11.2	11.6	12.1	13.2	15.0	15.1	16.0	
	J							4.0	4.1	4.5	5.0	5.5	6.3	7.2	8.8	9.0	10.0	11.9	11.5	13.0	14.0	
	M								3.8	4.6	4.9	5.0	5.9	6.5	7.9	8.2	8.9	10.0	11.2	13.0	14.0	
	I			1.4		1.8		2.2	3.0	3.6	4.0	4.7	5.1	5.8	6.6	7.3	8.0	8.9	9.2	11.5	12.5	
VIII, Set 3	D				2.8			4.3	4.9	5.7	7.0	7.9	7.5	8.6	10.0	11.0	11.9	13.2	13.8	15.0	16.0	
	J							3.3	3.8	4.1	5.5	6.3	6.8	7.2	8.1	9.5	11.1	11.5	10.1	12.3	13.0	
	M							2.6	2.7	3.6	4.7	5.2	6.1	7.1	7.3	7.3	8.5	9.5	11.0	12.5	15.0	
	I				1.3			4.8	5.0	5.5	6.7	8.0	7.1	7.5	8.0	8.0	7.9	9.0	10.0	12.0	12.8	
IX, Set 1	D								6.3	7.1	8.0	8.6	10.0	11.0	11.4	12.2	13.5	14.0	15.5	16.5	18.5	
	J									5.6	6.0	7.0	8.0	9.0	9.5	10.5	10.8	11.8	12.5	13.9	14.0	
	M				3.3		3.8	4.0	4.3	5.0	5.5	5.9	6.2	6.7	7.1	7.8	8.8	9.5	11.0	12.5	14.0	
	I						3.0	3.5	3.9	4.2	4.8	5.1	6.0	6.0	6.8	7.1	7.7	8.2	9.0	10.1	11.8	
IX, Set 2	D			3.0	3.5	3.8	4.7	5.0	6.5	6.8	7.6	8.1	9.0	11.0	11.0	11.6	13.0	14.9	14.0	16.0	15.0	
	J			2.4	2.8	3.3	3.5	3.8	4.4	4.8	5.4	6.1	7.0	8.2	9.2	9.2	10.2	11.9	12.5	14.5	15.5	
	M								3.1	3.9	4.6	5.0	5.5	6.2	7.0	7.4	8.6	10.0	11.1	12.6	14.0	
	I			2.1	2.4	2.9	3.0	3.4	3.9	4.8	4.9	5.5	5.8	6.2	6.7	6.5	7.5	8.7	10.0	11.2	12.9	
IX, Set 3	D	1.5		2.5	2.7	3.5			5.3	5.0	6.6	7.6	8.4	10.2	11.1	12.0	13.0	14.6	15.8	16.5	17.0	
	J	1.9		2.3	3.0	3.4			4.6	4.4	5.6	6.1	7.5	8.2	9.0	10.0	11.0	12.0	12.8	15.0	16.0	
	M			2.1	2.3	2.8			4.0	4.2	4.8	5.3	6.1	7.2	8.0	9.0	9.8	11.1	11.8	13.0	13.1	
	I	2.0		2.1	2.2	2.6			3.6	3.9	4.6	5.0	5.5	5.8	6.5	7.2	8.1	9.4	10.4	11.8	12.5	

temperature the rates decrease from the duodenum to the ileum. Since these differences are less at high temperatures than at low, the lines tend to converge with the temperature rise. This means that the acceleration for a given temperature rise is greater for the segments from the lower bowel than for those taken from the upper bowel. The fact that these data fall so nearly into straight lines indicates that they are following the law of Arrhenius for velocity of chemical reactions as affected by temperature. The simplest way to obtain the coefficient for increase in rate is to divide the rate at a certain temperature by the rate at 10° below. In obtaining the following figures, the rates were taken at the points where each of the four lines intersects the abscissae of 28° and 38°C.

Duodenum	2.42
Jejunum	2.48
Upper ileum	2.88
Lower ileum	2.68

This expresses mathematically what has been stated before, that the increase per unit of temperature rise is greater in the lower part of the bowel than in the upper. The rise in the early stages of warming was steeper with the upper three segments than with that from the lower ileum. It is interesting that work done by Moorehouse on strips from different parts of the mammalian heart showed similar differences in reaction to temperature changes (6). The curve of acceleration of the nodal strip usually showed a sharper ascent in the early stages than did the curves of the other strips. On the whole, he found the coefficient of acceleration highest in the septal and coronary strips which, in his series, correspond to the ileal segments in this work.

CONCLUSIONS

The evidence presented indicates that the rate of rhythmic contraction is determined by the chemical changes taking place in the muscle. The difference in the coefficient for the four different segments implies a difference in their metabolism.

Finally we wish to thank Dr. T. Brailsford Robertson, Dr. Donald Van Slyke and Dr. Robert Newell for assistance in the interpretation of the results.

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THE MECHANISM FOR VASODILATATION FROM ADRENALIN

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Up to the present, no satisfactory explanation of the vasodilatation resulting from adrenalin has been offered. That it might be of central nervous origin was indicated by the experiments of S. J. and C. Meltzer (1) in the dilatation of the vessels of the rabbit's ear from subcutaneous injection of adrenalin. They obtained nothing but constriction if the vasomotor nerves were cut. However the explanation of dilatation from subcutaneous injection of adrenalin in the rabbit is not necessarily the same for the cat because no dose of adrenalin causes a fall of blood pressure in the rabbit as it does in the cat.

Dale's (2) theory that small amounts of adrenalin stimulate vasodilator endings while larger amounts bring vasoconstrictors into play, is not supported by the observation that small amounts of adrenalin cause constriction while larger amounts cause dilatation of the intestine of the dog and cat (3). Nor does the hypothesis of Cannon and Lyman (4) satisfy the facts observed in the differential action of adrenalin (5). They attributed the two effects, vasodilatation and vasoconstriction to opposite actions according to the state of the muscle—relaxation when tonically shortened, contraction when relaxed.

Finally the work of Gruber (6) suggests that the central nervous system is involved in the dilatation from adrenalin. He found that doses of adrenalin which caused dilatation in the normal limb of the cat produced constriction when the nerves were cut.

In attempting to explain the dilatation from adrenalin in the intestine of the cat and dog we (3) found that if the splanchnics were cut constriction replaced dilatation. This observation led us into a study of the part played by the central nervous system in adrenalin vasodilatation. The following investigation is the result.

Ether anaesthesia was used in all experiments, except a few in which anocain was injected. Blood pressure in all except the anocain experi-

ments was taken from the right carotid artery while adrenalin was injected into the left external jugular vein. The limb volume was registered by means of Brodie's bellows connected to a glass cylinder enclosing the part. Intestinal volume change was also indicated by a bellows recorder connected to the oncometer. Adrenalin chloride (Parke, Davis and Company) was freshly prepared for each experiment by diluting with distilled water.

PROOF OF AN ADRENALIN VASODILATOR MECHANISM IN THE CENTRAL NERVOUS SYSTEM

1. By severing central nervous connection

a. In the intestine. Volume changes in the small intestine were studied by methods described in a former research (3). The amount of adrenalin necessary to cause dilatation of the intestine was first determined. Connection with the central nervous system was then severed by cutting the splanchnics or by removing the coeliac and superior mesenteric ganglia. This caused obliteration of the intestinal dilatation from adrenalin and in all except one case it was replaced by prolonged constriction. Three dogs and four cats were studied. Connection with the central nervous system seemed to be necessary for the production of the intestinal dilatation occurring with larger doses of adrenalin. We say larger doses because occasionally there is dilatation resulting from small doses, which may be accounted for by the relaxation of the intestinal walls ((3), p. 316).

b. In the limb. Although both dogs and cats were employed in the study of limb dilatation, because of the greater proportion of skin to muscle vessels in the limb of the dog, cats served our purpose best. Hoskins, Gunning and Berry (7) have shown that the skin acts by constriction to small doses of adrenalin.

Simultaneous records were made of the volume changes of the two hind limbs after one limb had been denervated. In denervation the femoral and sciatic nerves were cut as high up as possible through slits in the skin, which were sewn up immediately after. In this way the part played by the central nervous system in the dilatation from adrenalin could easily be shown. The two cylinders were of the same size and enclosed approximately an equal volume of limb. Moreover the cuffs of the plethysmograph were placed high up on the limb so that muscle predominated over skin.

Small doses of adrenalin usually gave dilatation in the normal limb and

constriction in the denervated limb. Large doses, such as those which usually produce a rise in blood pressure, caused constriction in both limbs. Ten cats out of fifteen gave this result. Four showed little or no change in the normal limb in response to small doses of adrenalin. That this failure of the normal limb to dilate might be due to a predominance of skin effects is not probable, for one of these limbs was skinned without modifying the reaction.[1]

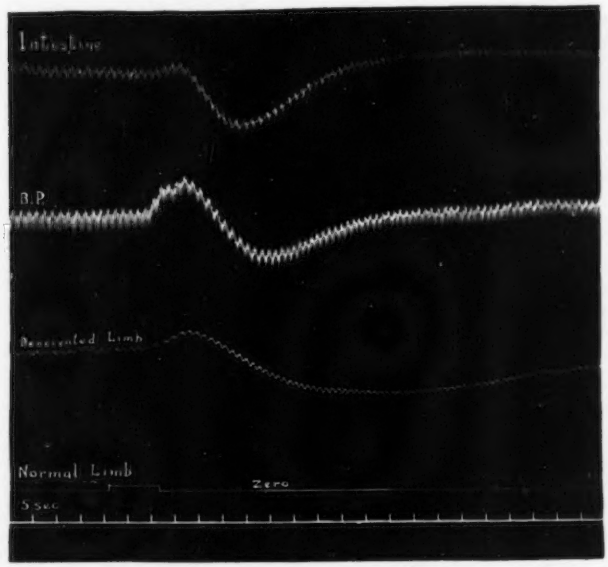

Fig. 1. Volume changes of normal limb, denervated limb and intestine in response to 0.6 cc. adrenalin, 1: 100,000. Cat weighing 3.3 kgms. (Reduced ⅓.)

The fifth cat (weight 4 kgm.) which was an exception as far as the response in the normal limb was concerned, was the most sensitive to adrenalin of any cat which has come under our observation. Even

[1] In order to determine the importance of the skin in volume changes in the cat's hind limb, we placed the two limbs of an animal which gave the typical limb dilatation in plethysmographs after one limb had been skinned excepting the sole of the foot. Dilatations from adrenalin were the same in both limbs. We concluded that if most of the hind limb was included in the plethysmograph, the skin reactions were negligible.

Fig. 2. Same as figure 1 in response to 2.0 cc. adrenalin, 1:100,000. (Reduced ⅓.)

0.025 cc. of adrenalin 1: 1,000,000 caused a fall of blood pressure from 125 mm. to 120 mm., while 0.2 cc., 1: 1,000,000 not only produced a fall of blood pressure from 127 mm. to 93 mm., but brought about constriction in both normal and denervated limbs. However, there is this to be said about these exceptional cases: the normal limb usually either gives no constriction or else much less constriction as compared to the denervated limb. So although there may be no positive dilatation there must be enough in the normal limb to lessen or else obliterate the local constrictor effect of adrenalin.

The following is a typical experiment of the ten which gave positive dilatation:

Cat, weight 3.3 kgms. (See figs. 1 and 2)

DOSE OF ADRENALIN cc. 1:100,000	BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY	NORMAL LIMB				DENERVATED LIMB			
		Dilatation		Constriction		Dilatation		Constriction	
		Amount	Duration	Amount	Duration	Amount	Duration	Amount	Duration
		cc.	*seconds*	*cc.*	*seconds*	*cc.*	*seconds*	*cc.*	*seconds*
0.2	118–127– 96	0.13	95	None		0.18	35	None	
0.4	118–131–100	0.33	178	None		0.16	31	None	
0.6	122–138–100	0.5	325	None		0.23	28	0.1	47
1.0	124–141–110	0.5		None		0.25	13	0.67	
1.5	127–153–117	0.34		None		0.15	11	0.9	275
2.0	127–157–113	0.13		None		0.10	9	1.4	
0.5 (1:10,000)	127–196– 89	0.13	18	0.63	74	0.12	5	1.45	265

The dilatation in the denervated limb was passive because it occurred at the same time that the blood pressure began to rise and lasted no longer than the rise. This dilatation was cut short or obliterated with larger doses of adrenalin. Dilatation in the normal limb came later than in the denervated limb and lasted as long as the blood pressure was below normal.

This difference in reaction between the denervated and normal limbs is not due, as Gunning (8) suggests, to extreme dilatation of the denervated limb resulting from the absence of vasoconstrictor impulses. A depressor substance obtained from ox pituitaries was injected into the animal that produced figures 1 and 2. The dilatation of the denervated limb was as great as that in the normal limb, showing that the limit had not been reached.

In one dog (17 kgm.) a plethysmograph was placed so as to include a hind limb just above the ankle. Adrenalin was injected into the circulation. As a result the limb was constricted as much as 0.66 cc. in one instance and as little as 0.3 cc. in another. Without disturbing the plethysmograph both femoral and sciatic nerves to the enclosed limb were then severed. The same amount of adrenalin (0.2 cc., 1: 10,000) was injected as before with a resulting constriction of 1 cc. or more in every instance. Moreover the duration of constriction in the dener-

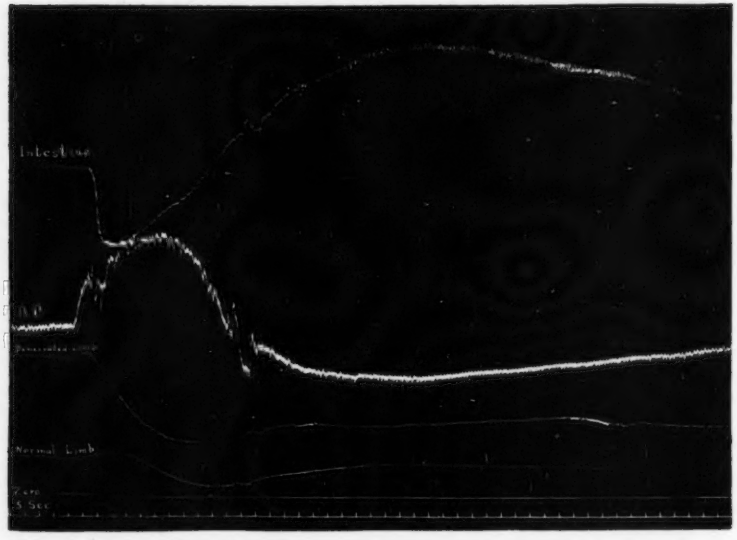

Fig. 3. Same as figure 1 in response to 0.5 cc. adrenalin, 1:10,000. (Reduced ⅔.)

vated limb was almost twice as long as that before cutting central nervous connection (fig. 4). The greater and more prolonged constriction, after the nerves were cut, was no doubt due to absence of dilatation from central nervous influence. This explanation also probably applies to one of the cats which failed to give dilatation in the normal limb, for the constriction in the normal limb was always decidedly less than in the denervated limb.

We attempted to demonstrate the necessity of central nervous connection for dilatation from adrenalin in another way. The dose which

gave the maximum fall in blood pressure was determined. A number of injections were made so that the average might be found. After cutting the nerves to the limbs the percentage fall in blood pressure from

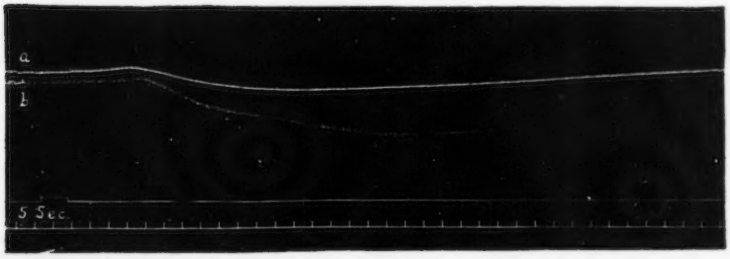

Fig. 4. Increase of constriction in a dog's foot by destroying central nervous connection. *a*, Before cutting nerves; *b*, after cutting nerves (0.2 cc. adrenalin, 1: 10,000; weight 17 kgms.). (Reduced ½.)

the same dose was ascertained. According to the evidence just submitted the percentage fall should decrease with every limb denervated.

In the first animal, a cat weighing 1.3 kgm., 0.2 cc. adrenalin, 1: 100,000 caused a fall in blood pressure of 18.7 per cent (from 123 mm.). After cutting the left brachial plexus the same dose produced a fall in blood pressure of 12.2 per cent, (from 114 mm.). Cutting the right brachial plexus reduced the fall to 9 per cent (from 100 mm.).

A cat weighing 2.3 kgms. responded to 0.1 cc. adrenalin, 1: 100,000 by an 8 per cent fall in blood pressure (from 150 mm.), and to 0.3 cc. adrenalin, 1: 100,000 by an average fall of 16.6 per cent (4 injections). When both brachial plexuses and both femoral and sciatic nerves had been cut the percentage fall from 0.1 cc. adrenalin, 1: 100,000 was 2.7 while from 0.3 cc. adrenalin, 1: 100,000 the average fall (4 injections) was 9.3 per cent (from 112 mm.), (see fig. 5).

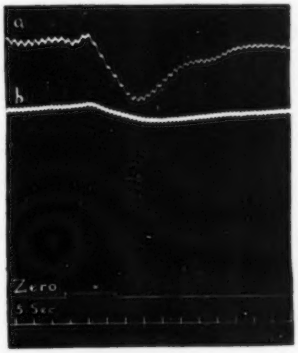

Fig. 5. Blood pressure reactions to adrenalin (0.3 cc., 1: 100,000) before and after cutting the nerves to the limbs. *a*, Before; *b*, after. Cat weighing 2.3 kgms. (Reduced ½.)

The above doses were so small that the intestine would scarcely contribute to the fall in blood pressure. After dilatation in the limbs had been prevented by destroying the central nervous connection there still remained the important back, chest, shoulder and hip muscles, which easily accounts for the large percentage fall still obtained. On the whole, this evidence tends to support our plethysmographic proof of a central nervous function in adrenalin blood pressure fall.

2. By perfusion experiments

A conclusive method of proving that the dilatation from adrenalin is due to a central nervous mechanism was devised. The part to be studied, limb or intestine, was cut off from the body circulation and perfused with warm oxygenated Ringer's solution. The nerves to this part were left intact. Dilatation of the part was ascertained by either the variation in outflow of the perfusing fluid or by means of a plethysmograph. Injections of adrenalin were then made into the body circulation and their effect upon the organ in question noted.

The perfusion fluid, oxygenated Ringer's solution or defibrinated blood mixed with Ringer's solution, was warmed by passing it through a tube enclosed in a cylinder through which warm water circulated. The apparatus was a modification of that described by Brodie and Cullis (9). A small thermometer inserted into the solution, by means of a T-tube, just before it entered the perfusion cannula enabled us to know the temperature as it started through the organ. Pressure was produced by compressed air led into the bottle containing the perfusion fluid. The pressure was controlled by means of a mercury valve.

The temperature of the liquid as it actually entered the organ was maintained in the neighborhood of 37°C. The pressure was usually somewhat below the general blood pressure.

To perfuse an intestinal loop without injuring its nerves large animals were almost necessary. A loop of intestine of a dog weighing 15 kgms. was perfused with a mixture of defibrinated blood and warm Ringer's solution after the circulation had been entirely cut off. The perfusion pressure was 120 mm. of mercury. The intestine was then enclosed in an oncometer. Injection of 0.5 cc. adrenalin, 1:10,000 into an external jugular vein caused a rise and fall in the blood pressure (136 mm. to 156 mm. to 93 mm.) and a dilatation in the intestinal loop which could in no possible way be reached by the adrenalin itself. A larger injection of adrenalin 2.0 cc., 1: 10,000, caused a rise in blood pressure (97

mm. to 172 mm.) and a marked dilatation of the intestine. The dilatation of the intestine was accompanied by an increased outflow of the perfusion fluid.

We next perfused one hind limb of another dog (5.4 kgms.). The perfusion fluid was forced into the femoral artery and any change in the limb vessels was determined by measuring the outflow from the femoral vein. An injection of 0.2 cc. adrenalin, 1: 10,000 caused an increase in the outflow from the femoral vein. In order to cut off the rest of the limb from the body circulation nearly all of the muscles were cut down to the bone, care being taken to leave the nerves intact. The same dose of adrenalin produced a decrease in the outflow. Apparently some adrenalin was still reaching the limb thus causing constriction and the vasodilator mechanism was perhaps inhibited by the vigorous operation of cutting the muscles.

The intestine and limb of a third dog (23.5 kgms. in weight) were perfused. The intestine employed was 20 cm. long. It could not be influenced directly by the adrenalin injected as it was entirely cut off from the body circulation; 0.7 cc. adrenalin, 1: 10,000, injected into the jugular vein caused a dilatation of 0.28 cc. in the perfused intestinal loop; 3 cc. adrenalin, 1: 10,000 into the general circulation produced a dilatation of 0.88 cc. in the loop. There was also a measurable increase in the venous outflow from the intestine.

One hind limb of this dog was include in a plethysmograph. The cylinder was large enough to include a major portion of the limb. Before its circulation was interfered with, 0.5 cc. adrenalin 1: 10,000 injected into the general circulation caused a dilatation of 0.86 cc. (see fig. 6). Cannulae were inserted into the iliac artery and vein just below the bifurcation. The aorta was clamped considerably above the iliacs in order to prevent access of blood to the perfused limb by anastomoses. Ringer's solution at a temperature of 37°C. entered the limb by way of the iliac artery under pressure of 100 mm. of mercury. The same amount of adrenalin as before was injected into the general circulation. The first time the perfused limb dilated 0.68 cc. while at the second injection the expansion was 0.90 cc. In other words the amount of dilatation was about the same when circulatory connection was destroyed as when it was intact (see figs. 7 and 8).

The amount of dilatation varied with the dose; 0.25 cc., 1: 10,000 gave 0.27 cc., while 1 cc., 1: 10,000 gave 1.59 cc. After the limb had been perfused one and one-half hours, 1.0 cc. adrenalin, 1: 10,000 into the jugular vein gave only 0.72 cc. dilatation. Another interesting

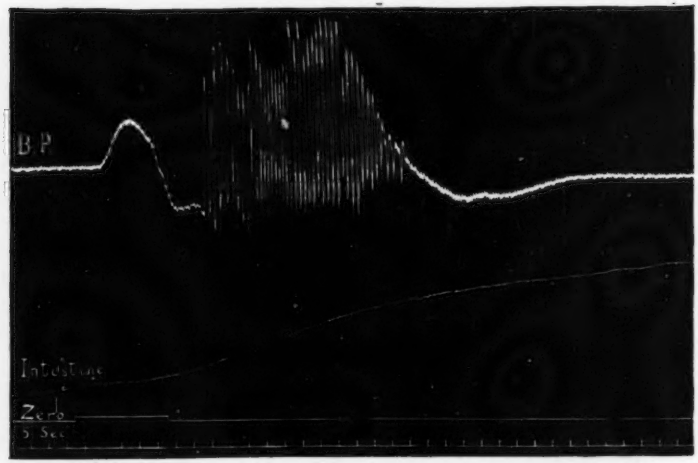

Fig. 6. Dilatation of a perfused loop of intestine, which has no connection with the body circulation, in response to adrenalin (3.0 cc., 1: 10.000) injected into the jugular vein. Nerves to intestine undisturbed. Dog weiging 23.5 kgms. (Reduced ½.)

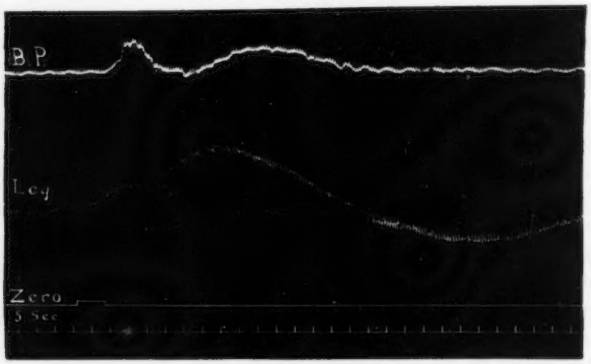

Fig. 7. Dilatation of a dog's hind limb in response to 0.5 cc. adrenalin, 1: 10,000. Dog weighing 23.5 kgms. (Reduced ½.)

fact was the persistence of dilatation in the perfused limb. The intact limb returned to normal in sixty-five seconds when a dose of 0.5 cc. adrenalin, 1: 10,000 was given, while the same limb perfused returned very slowly or not at all after a similar dilatation from adrenalin.

Thus we have proven both by cutting the nerves and by destroying the circulatory connection that the dilatation from adrenalin, in the intestine and in the limb, is due to some central nervous mechanism.

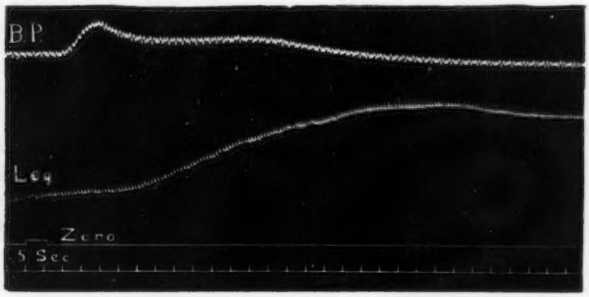

Fig. 8. Response of same limb as in figure 7, after connection with the body circulation has been destroyed. Perfused with oxygenated Ringer's solution. Nervous connection intact. Adrenalin (0.5 cc., 1: 10,000) injected into jugular vein. (Reduced ½.)

INHIBITION OF THE ADRENALIN VASODILATOR MECHANISM

Although only about 5 per cent to 10 per cent of cats and dogs fail to give a fall in blood pressure with small doses of adrenalin, many of those animals are not normal in other ways. In a former study ((5) p. 443) three out of five such cases were diseased or emaciated while a fourth had been intoxicated with alcohol a few hours before. More recently we have investigated a case (cat weighing 2.4 kgms.), in which there was either only a slight fall or else no fall in blood pressure with doses of adrenalin as small as 0.1 cc. or 0.2 cc., 1: 1,000,000. The intestine was placed in an oncometer and gave dilatation after the injection of 0.5 cc. adrenalin, 1: 10,000 into the circulation. So in spite of the fact that there may be no definite fall in blood pressure, dilatation of the intestine may occur.

These observations kept us on the lookout for other conditions which might inhibit the adrenalin vasodilator mechanism. We found a cat

weighing 4.3 kgms. which gave a fall in blood pressure of only 6 mm. (from 113 mm.) with 0.6 cc. adrenalin, 1: 100,000. At the same time there was little or no effect on the volume of the normal limb. Associated with this poor response was a very dark venous-colored blood in the arteries. There had been no other signs of asphyxia. A plug of mucus in the trachea was found. Several minutes after the restoration of ventilation by removal of the mucus 0.5 cc. adrenalin, 1: 100,000 produced a fall in blood pressure of 38 mm. from 140 mm. with dilatation of the normal limb. In this animal partial asphyxia seemed to produce incomplete inhibition of the adrenalin vasodilator mechanism.

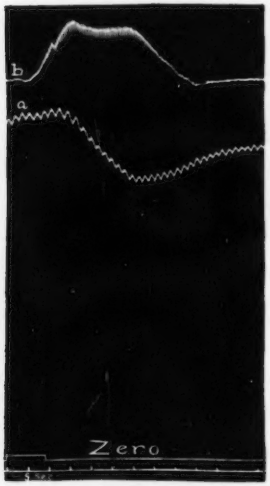

Fig. 9. Reversal of blood pressure response to 0.5 cc. adrenalin 1: 100,000, after injecting anocain. *a*, Before injecting anocain; *b*, after injection of anocain. Cat weighing 2.2 kgms. (Reduced $\frac{2}{3}$.)

We had occasion to employ anocain (manufactured by Wingate Chemical Company, Ltd., Montreal) as a local anaesthetic in taking blood pressure from a dog's femoral artery. Injections of adrenalin in doses which in other animals gave a fall in blood pressure caused either no effect or only a rise in blood pressure. In order to determine whether the abnormal response was due to anocain a cat (weight 2.2 kgms.) was gently fastened to the board without its being alarmed. Then a solution of anocain (0.05 gram) was injected into the skin and later the muscles on either side of the femoral artery. Adrenalin, 1: 100,000 was injected into the femoral vein. Blood pressure was registered from the femoral artery; 0.1 cc. caused a rise of 15 mm. from 180 mm.; 0.5 cc, increased the blood pressure 26 mm. from 192 mm., (see fig. 9). Following this the cat was placed under the influence of ether. Thirty minutes after the above reaction with anocain anaesthesia, 0.1 cc. adrenalin produced a fall of 10 mm. in blood pressure (from 175 mm.) while 0.5 cc. decreased the blood pressure 24 mm. (from 173 mm.).

One hour after anocain had been given, 0.5 cc. adrenalin produced a fall of 33 mm. (from 175 mm., see fig. 9). Anocain was again given intramuscularly after which adrenalin produced nothing but a rise in blood pressure.

After sufficient time had elapsed the fall in blood pressure again returned upon the injection of the usual dose of adrenalin.

This experiment was repeated in another cat with similar results. We would conclude that anocain inhibits the action of the adrenalin vasodilator mechanism.

There is some evidence that dilatation of a limb may be prevented by afferent impulses from that part. Many of the experiments which gave no dilatation from adrenalin in the normal limb were performed in a cold laboratory (15°C. or less). Although the operating table was warmed, the limbs were never as warm as the back of the animal. Because the temperature was much lower than in most of our successful experiments we thought that it might be the cause of a local inhibition of dilatation in the limb. Dilatation from adrenalin was occurring some where in the animal for there was a marked fall in blood pressure with the usual dose. The intestine was not dilating because the dose of adrenalin was below the intestinal dilatation threshold, moreover the loop of the intestine in the oncometer was constricting. We assumed that the dilatation must take place in the muscles which were kept warm, such as the back muscles. To investigate this assumption we warmed the normal limb with heat from an electric lamp. After the limb had ceased dilating from the artificial heat the usual dose of adrenalin injected into the general circulation caused an increase in volume of the limb. Previously the same doses of adrenalin produced either no effect or else constriction in the same limb.

It seems also that cutting the muscles in a limb may inhibit the dilatation from adrenalin. In one of the first perfusion experiments mentioned above, the response to the usual dilator-dose of adrenalin was changed to constriction by cutting part of the muscles of the thigh although the nerves were not injured.

BEHAVIOR OF THE ADRENALIN VASODILATOR MECHANISM

Simultaneous records of the volume changes of the intestine, normal hind limb and denervated hind limb together with the blood pressure in response to adrenalin, were obtained from thirteen cats. Whether the threshold dose for constriction in the normal limb was also the threshold dose for dilatation in the intestine could be answered in this manner.

Our apparatus showed that the threshold dose for dilatation in the limb was a little larger than the smallest dose causing a blood pressure

fall, while the threshold dose for intestinal dilatation was usually four or five times as large as the threshold dose for the limb. Nor does the intestinal dilatation reciprocate the action of the normal limb for the latter usually continues to dilate some time after the threshold dose for dilatation of the former has been reached. Moreover there is an important distinction in the behavior of the limb and intestine. If we look at the effects on these respective parts with increasing doses we see that although the dilatation of the limb increases with increasing doses up to a certain point, beyond that the dilatation becomes less and less until constriction results. On the other hand constriction is the first adrenalin effect in the intestine, while with increasing doses dilatation appears and becomes larger and larger but is never replaced by constriction after the maximum is reached. Stated in another way, small doses of adrenalin act on the adrenalin vasodilator mechanism for the limb while large doses act on the adrenalin vasodilator mechanism for the intestine. Figures 1, 2 and 3 picture the results with increasing doses of adrenalin. The following experiment illustrates the foregoing statements:

Cat, weight 3.3 kgms.

DOSE OF ADRENALIN 1:100,000	BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY	NORMAL LIMB		DENERVATED LIMB		INTESTINE	
		Dilatation	Constriction	Dilatation	Constriction	Constriction	Dilatation
cc.		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
0.2	126–135–107	0.13			0.2	0.23	
0.6	124–140–106	0.26		0.15	0.6	0.5	
1.0	125–141–112	0.25		0.10	0.55	0.18	1.0
2.0	132–153–119	0.20		0.10	0.85	0.63	1.22
0.5 (1:10,000)	127–196– 89	0.13	0.63	0.12	1.45	1.22	2.34

AN INTERPRETATION OF THE ERGOTOXINE REVERSAL OF ADRENALIN ACTION

Dale (10) has shown that ergotoxine inhibits the action of the endings of constrictor fibers. Instead of assuming as Dale suggests that the fall in blood pressure from pressor doses of adrenalin after ergotoxine is due to stimulation of vasodilator endings, in the light of our present research we would interpret it in another way. We suggest that the fall is due to stimulation of the central vasodilator mechanism by adrenalin and failure to stimulate the constrictor endings because of their

paralysis by ergotoxine. While constrictor endings function normally their total effect is greater than that of the vasodilator mechanism when pressor doses of adrenalin are injected. In a cat we were able to obtain evidence in support of this interpretation. We first determined the response of normal limb, denervated limb and intestine to adrenalin. Ergotoxine phosphate was given until the denervated limb which previously had given constriction, gave no response to adrenalin. The normal limb still responded by dilatation. With larger doses of adrenalin the intestine dilated also.

There is additional evidence that our interpretation of ergotoxine reversal is correct, in the failure to obtain a reversal in the rabbit after ergotoxine (10). Unpublished work from this laboratory shows that the rabbit does not possess the adrenalin vasodilator mechanism; that is, adrenalin will not cause vasodilatation in the limb or in the intestine.

LOCATION OF THE ADRENALIN VASODILATOR MECHANISM

We have proven by experiment that destruction of the cerebrum does not prevent the vasodilatation from adrenalin.

A cat (2.8 kgms.) gave a fall in blood pressure of 29 mm. from 150 mm. with 0.2 cc. adrenalin, 1: 100,000. After removal of the cerebrum the same dose produced a fall of 22 mm. from 114 mm. Further investigation is being carried on as to the location of the adrenalin vasodilator mechanism.

We have been able to show that the central nervous system is necessary for active dilatation resulting from adrenalin. Just how this mechanism is brought into action, it is impossible to say at the present time. The use of such a mechanism is obvious in case of muscle, but the object of vasodilatation in the intestine is not evident.

SUMMARY

1. Adrenalin vasodilatation in the intestine and limb is prevented by cutting the nerves to these organs.

2. A perfused limb or intestinal loop with nerves intact but without circulatory connection will dilate when the appropriate amount of adrenalin is injected into the general circulation.

3. Ill health, asphyxia, alcohol or anocain may inhibit the action of the adrenalin vasodilator mechanism.

4. Afferent impulses such as result from cooling or from cutting the muscles of a limb may impair or inhibit the adrenalin dilator reaction in that part.

5. The dilatation from adrenalin in a normal limb is produced by smaller amounts of adrenalin than those which cause intestinal dilatation.

6. Limb dilatation is replaced by constriction if the adrenalin is in sufficient quantity.

7. Intestinal dilatation is never replaced by constriction if the dose is above the dilatation threshold.

8. Ergotoxine reversal of adrenalin pressor effects is accounted for by paralysis of the vasoconstrictors which mask the adrenalin vasodilator mechanism when large doses of adrenalin are used.

9. The adrenalin vasodilator mechanism is not located in the cerebrum.

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STUDIES IN THE PHYSIOLOGY OF THE RESPIRATION

II. The Changes Taking Place in the Composition of the Alveolar Air During Inspiration and Expiration

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There has been a great deal of discussion recently concerning the mean percentage composition of the alveolar air and the capacity of the dead space. This is due to the fact that all the existing methods for the determination of the gaseous composition of the alveolar air have not yielded results that are above reproach. The direct method for sampling developed by Haldane and Priestley (1) has given excellent results in elucidating many of the problems of respiration, and is the most accurate of all direct methods, but it contains some theoretical as well as practical errors which leave much to be desired in obtaining absolute values for the composition of alveolar air. The indirect methods which are calculated from the percentage of CO_2 in the expired air assume a knowledge of the capacity of the dead space. Methods for the calculation of the dead space are also inaccurate or at least do not give identical results. For example, Haldane and Douglas, Haldane and Henderson and their coworkers have found that the dead space varies greatly during deep breathing. This view has been seriously questioned by Krogh and Lindhard (2) and also recently by me (3) in papers appearing at the same time. Because of the great amount of criticism which has been leveled against these methods, I have sought to learn the various factors which may influence the composition of the alveolar air and the capacity of the dead space, and from these considerations to produce a method for the determination of the capacity of the dead space and the composition of the alveolar air in which these factors are taken into account, thus reducing errors to a minimum.

In normal breathing which is regulated to the needs of the body, the relationship of the CO_2 excretion to the O_2 intake is expressed by the

respiratory quotient, which over limited periods of time is a comparatively constant factor. There are two contingencies which may change the quantity of O_2 or CO_2 in the blood providing the type of metabolism remains the same. These are (*a*) or (1), a change in the mass movement of the blood, and (*b*) or (2), a change in the pulmonary ventilation. In the case of O_2 an increased ventilation cannot greatly increase the amount of O_2 in the blood without a commensurate increase in its mass movement, for at ordinary pressures there is always an excess of the O_2 pressure over the optimum for actual body needs, and the blood leaves the lungs at all times practically saturated with O_2. If the circulation rate be increased, the blood will return to the lungs retaining a larger percentage of its total O_2 than usual. If the circulation be slowed, the converse will hold.

In the case of CO_2, however, the conditions are different, for any increase in the pulmonary ventilation will allow an increased escape of the gas into the alveolar air and a consequent decrease in the CO_2 tension in the blood; conversely, a decrease in the pulmonary ventilation causes a piling up of CO_2. If the mass movement of blood is decreased and the production of CO_2 in the body is unchanged, then each unit of blood must carry back to the lung a greater volume of CO_2 than under normal conditions. Such an increase in the tension of CO_2 in the *venous* blood stimulates the respiratory centers, and the minute volume of the respiration is increased. The tension of CO_2 in the alveolar air and *arterial* blood must then be reduced below normal in order to permit the decreased amount of blood to absorb the CO_2 produced by the tissues without any marked increase in the CO_2 tension of the venous blood. We have shown this to be the case in congenital heart disease and some cases of broken compensation (4). We have attempted to make a detailed examination of the events of the respiratory cycle, taking into consideration only the point over which there is at present no contention. In doing this we make use of data obtained from the experiment the protocol of which is as follows:

The subject breathed into a large Krogh spirometer for nine breaths, and the tenth expiration was deepened and collected in a small Krogh spirometer (see details of technique on page 385). The volume of air expired at each normal expiration, the volume of air expired at the deep expiration and the time taken for these procedures, together with the analysis of the air expired in each spirometer, were determined. From these data calculation was made, by the binomial formula, of the true average respiratory quotient for the period, of the O_2 absorbed

and CO_2 excreted per respiratory cycle, and of the composition of the alveolar gases and the dead space (see page 377). The tracing obtained in this experiment is that shown by figure 1, the respirations being regular in rate and depth throughout, and the time of the deep expiration approximately the time occupied by normal expirations.

Samples of air were taken for the sake of comparison by the Haldane-Priestley method at the end of a forced expiration following a normal inspiration (no. 1), and at the end of a forced expiration following a normal expiration (no. 2), and a sample of air was taken at the end of a normal expiration. This was obtained by breathing normally through a soft rubber tube equipped with an expiratory valve about 10 cm. from the mouth piece. At the end of a normal expiration, the mouth

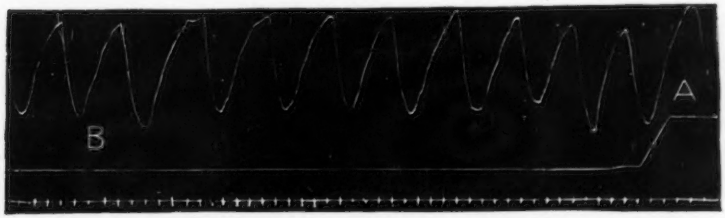

Fig. 1. Upper line: pneumographic tracing. The expriations from *B* to *A* were collected in the large spirometer. Middle line: tracing obtained by the movement of the small spirometer when expiration *A* was collected. Lower line: time in seconds.

piece was closed by the tongue and a 10 cc. sample of air withdrawn through a side tube from the space between the tongue and the expiratory valve.

Data. Subject R. G. P. Time 3.30 p.m.; temperature 22°; barometric pressure, 747 mm.; 4890 cc. of air were expired in 9 expirations and in 43 seconds. The volume of the tidal air was therefore 543 cc., and the time of the respiratory cycle was 4.8 seconds.

Argument. From the above it is seen that 23 cc. of CO_2 are excreted during the respiratory cycle; and this amount would serve to increase the percentage of CO_2 in the alveolar air to 6.30 per cent, if no inspiration occurred $\left(\frac{2300 \times 0.053 + 23}{2300} = 6.30\right)$. To prevent this excessive increase of alveolar CO_2, it is necessary to dilute the 2300 cc. with 435

cc. $\left(\frac{2300 \times 0.063}{0.53} - 2300 = 435\right)$. Subtracting 435 from 543 gives a dead space of 108 cc. The dead space calculated by means of the binomial formula is 83 cc. for CO_2 and 103 cc. for O_2. The discrepancy between 86 and 108 lies, no doubt, in the assumption that 2300 cc. is the correct figure for residual and supplemental air; and the other individual determinations are also subject to experimental error. Now, if the rate of discharge of CO_2 is equal throughout the respiratory cycle, and the inspiratory and expiratory phases are of the same length, the lungs will contain 11.5 cc. more CO_2 at the end of inspiration than at the

TABLE 1

	AVERAGE EXPIRATION. LARGE SPIROMETER*	LARGE EXPIRATION. SMALL SPIROMETER*	COMPOSITION ALVEOLAR AIR*	HALDANE-PRIESTLEY SAMPLE†		AIR AT END OF EXPIRATION†
				NO. 1	NO. 2	
Volume	543	790				
Per cent CO_2	4.24	4.49	5.040	5.100	5.620	5.300
Per cent O_2	16.09	15.75	15.020	14.600	13.950	14.350
Per cent O_2 absorbed	5.04	5.40	6.610	6.640	7.470	6.900
Respiratory quotient	0.84	0.831	0.818	0.777	0.750	0.770

* Mean from two sets of determinations.

† Mean from ten sets of determinations. Greatest variation from mean was ± 0.05 per cent for CO_2.

CO_2 excreted per respiratory cycle (4.8 seconds) = 4.24 × 5.43 = 23 cc., or 282 cc. per minute.

O_2 absorbed per respiration in 4.8 seconds = 5.04 × 5.34 = 27.4 cc.

Residual and supplemental air by O_2 method = 2300 cc.

Dead space for CO_2 = 83 cc.; for O_2 = 103 cc. Average = 93 cc. (binomial formula.)

beginning of expiration. This would bring the percentage of CO_2 up to 5.82 per cent. $\left(\frac{2300 \times 0.053 + 11.5}{2300} = 5.82\right.$, had there been no dilution.) However, it is during this phase of respiration that the total diluting air is added (450), which would bring the percentage of CO_2 down to 4.85 per cent. $\left(\frac{2300 \times 0.053 + 11.5}{2300 + 450} = 4.85\right.$, at the end of inspiration, or a tension of 34 mm. of Hg., the barometric reading being corrected to room temperature, dry.)

The blood in the pulmonary capillaries is therefore under a less tension of CO_2 and greater pressure of O_2 during inspiration than during ex-

piration. If the same variation in the tensions of the alveolar gases were to be kept up during inspiration and expiration, it would be necessary for one-half of the alveolar tidal air to be added during inspiration and one half during expiration.

The dissociation curve for CO_2 in the blood, as given by Christiansen, Douglas and Haldane (5), shows that CO_2 is given off relatively more rapidly at low partial pressures of CO_2 and in the presence of an abundance of O_2, than at high partial pressures of CO_2 and low partial pressures of O_2. This being the case, we must believe that the rate of evolution of CO_2 from the blood is more rapid during inspiration than during expiration, and that the blood which is in the capillaries of the lungs during inspiration contains less CO_2 than the blood in the capillaries during expiration. Hence we conclude that in a respiratory cycle in which the inspiratory and expiratory phases are equal in time, a greater percentage of the CO_2 excreted leaves the blood during the inspiratory phase, and that the speed of the increase in the alveolar CO_2 tension during inspiration due to the evolution of blood CO_2 is accelerated over that present during expiration.

In attempting to show this graphically, we have determined the points on the curve which Christiansen, Douglas and Haldane give for the dissociation of CO_2 in the blood in the presence and absence of O_2, which represent the tension and volume of blood CO_2 corresponding to calculated alveolar CO_2 tensions at: *a*, end of inspiration (34.5); *b*, end of expiration (37.1); and the mean as calculated by the binomial equation (35.22). The lower curve gives the dissociation of CO_2 in Haldane's blood in presence of O_2; the upper in completely reduced blood. Following the description of these authors, we have determined the line (fig. 2) representing the dissociation of CO_2 at all possible concentrations of oxyhaemoglobin, as in the experiment a respiratory quotient of 0.83 would allow the blood to return with an increase of 15 volumes per cent more of CO_2 in completely reduced blood, providing all the O_2 was used in the tissues, than is present in blood containing, at the average alveolar CO_2 tension, 96 per cent oxyhemoglobin. The value was, hence, determined on the upper curve, and this point connected with the point on the lower curve which represents the tension of CO_2 in the blood as it leaves the heart (35.28 mm. Hg.). This line then shows the average dissociation curve in the body. We have determined the venous CO_2 tension of the same subject under the same physical condition, and found it to be 42 mm. Hg. At this tension it is seen that the blood would contain 54 volumes per cent CO_2. At 35.28 mm. Hg., which represents

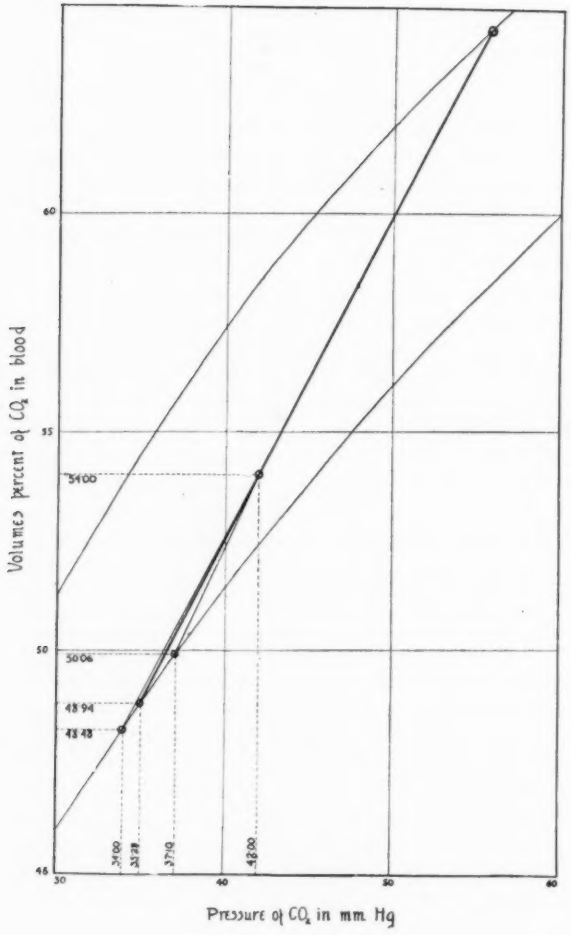
Volumes percent of CO_2 in blood
60
55
50
45
5400
5006
4894
4848
3900
3828
3719
4800
30
40
50
60
Pressure of CO_2 in mm. Hg

Fig. 2

the mean tension of CO_2 in the alveolar air, the blood contains 48.94 volumes per cent CO_2. At the tension of CO_2 present in the alveolar air at the end of an inspiration, i.e., the lowest calculated tension (34.0 mm.), it would contain 48.48 volume per cent CO_2. In other words, each 100 cc. of blood in passing through the lungs loses CO_2 according to the amount of oxyhaemoglobin present and the partial pressure of CO_2 in the alveolar air. For example, at 37.1 mm. of CO_2 (5.3 per cent pressure present at the end of expiration), it would require 584 cc. of blood to deliver the 23 cc. of CO_2 excreted during the respiratory cycle; whereas, if the CO_2 were discharged at the lowest pressure, 34 mm. Hg. (4.85 per cent), only 411 cc. would be required. Again, 454 cc. of blood would be necessary to deliver the 23 cc. of CO_2 at the mean alveolar CO_2 tension. Now, it is evident that since the mean pressure is much nearer the lowest figure than it is to the tension present at the end of expiration, the CO_2 must be evolved with progressively greater difficulty as the respiratory phase progresses. From these figures it is an easy matter to calculate the percentages of CO_2 evolved above and below the mean pressure of CO_2; namely, 75.1 per cent and 24.9 per cent, respectively.

The figures were obtained by the following calculation:

54. $-50.06 = 3.94$ cc. Volume per cent CO_2 lost by blood in capillaries at end of expiration

54. $-48.48 = 5.52$ Volume per cent CO_2 lost by blood in lungs at calculated pressure present in the lungs at the end of inspiration

54. $-48.94 = 5.06$ Volume per cent CO_2 lost by the blood in the lungs at mean pressure of CO_2 in the alveolar air

Since 23 cc. of CO_2 was excreted each respiratory cycle it would require $23 \div 3.94 \times 100$ or 584 cc., to evolve this much gas at the pressure present in the lungs at the end of expiration. Likewise $(23 \div 5.52 \times 100)$ 411 cc. of blood would be required to evolve 23 cc. of CO_2 at the pressure present at the end of inspiration; or again $(23 \div 5.06 \times 100)$ 454 cc. at the mean pressure of CO_2 in the alveolar air.

But if we assume the blood flow is constant, then more gas would be evolved at pressures below normal than above normal, the amount being determined as follows:

$$584\text{–}454 = 130 \text{ and}$$
$$454\text{–}411 = 43;$$

i.e., for each 43 cc. of gas given off at pressures above the mean, 130 cc., 24.9 and 75.1 per cent respectively, must be given off at pressures below the mean.

This result is, as expected from the theoretical consideration, i.e., because the entire influx of diluting air occurs during inspiration—and from

the effect of oxyhemoglobin on the driving off of CO_2. However, the difference in this latter effect between inspiration and expiration is very slight where O_2 is always in excess, as it is at ordinary barometric pressure, but at low tensions of O_2 the effect of O_2 is considerably greater during inspiration than during expiration. The importance of this will be shown in a following paper. It must be assumed that the tension of CO_2 in the alveolar air never reaches the low level calculated (4.85 per cent), which assumes that one-half of the CO_2 excreted comes off during inspiration. We have no means at present to determine just how much more is evolved during inspiration than expiration, and therefore cannot calculate just what the lowest tension of CO_2 reached during inspiration is. It must lie, however, between 4.85 and the mean alveolar CO_2 tension, which we found experimentally to be 5.04 per cent. This latter figure is near to the general average value of CO_2 in alveolar air for R. G. P., as estimated by the binomial formula under similar physiological conditions.

Since this forms a basic figure in the experiment, we must inquire into the theoretical soundness of the binomial formula for the determination of the percentage composition of alveolar air. This point has been questioned recently by Henderson and Morriss (6), but they fail to support their criticism with any attempt at an analysis of the method.

If we may assume that the percentage of CO_2 is least at the end of an inspiration and greatest at the end or shortly following the end of an expiration, and that the rate at which the air is taken in during inspiration equals that at which it is given off during expiration, then it must follow that the volume of CO_2 which is evolved below any pressure between the minimum and the maximum during expiration produces the same changes in the partial pressure of CO_2 in the alveolar air as does the volume of CO_2 which is evolved during inspiration below the same pressure, and that the changes in pressure produced by the CO_2 evolved above this point are equal for inspiration and expiration. In this assumption we must also include the pretty well established fact that the tension of CO_2 in the blood leaving the lungs at any time is in equilibrium with the partial pressure of CO_2 in the alveolar air. At this point it may be well to recall the fact that a larger volume of CO_2 must be given off by the blood during the period of inspiration to make the same pressure changes but in the reverse order to that of expiration, when there is a decreasing volume of air in the alveoli.

If, following normal expiration, we make in one case an expiration of normal length and depth and in another an expiration of normal length but of greater depth, then the percentages of CO_2 or of O_2 in the expired alveolar air of these expirations will be approximately equal, and the percentages of CO_2 or of O_2 in the total expired air of the two expirations will differ only as the dead space exerts a greater diluting effect in the small than in the large expiration. If the assumptions are made that the capacity of the dead space in the two respirations is alike, and that the percentage composition of the alveolar air is constant under the same physiological conditions from minute to minute, then the capacity of the dead space and the percentage of CO_2 or O_2 in the alveolar air can be determined by combining the data of the two expirations in a formula as follows.

Let A = amount of air in large expiration,
Ai = amount of air in small expiration,
B = the percentage of CO_2 or O_2 in the expired air of large expiration,
Bi = the percentage of CO_2 or O_2 in the expired air of small expiration,
x = the capacity of the dead space,
y = the average percentage of CO_2 or O_2 in the alveolar air; then,
$AB = (A - x)y$ and
$AiBi = (Ai - x)y$.

Solving this for x, y remaining constant under the same physiological conditions, and B and Bi when solving for the dead space for O_2 equaling the O_2 absorbed, we have: $x = \frac{AAi \quad (B - Bi)}{AB - AiBi}$, the dead space. Or solving for y we have: $y = \frac{AB - AiBi}{A - Ai}$, the mean percentage of CO_2 or O_2 in the alveolar air.

In these formulas we must assume that the dead space and the percentage composition of the alveolar air are alike under the two conditions. Is this allowable? For the present we will not take up the question of variations which undoubtedly occur in the capacity of the dead space, due to rhythmical contractions of the bronchial musculature, or that which may occur when the chest is expanded with unnatural breathing. These will be considered later. The method we have

adopted for the taking of the total expired air we believe makes it very improbable that variations in the size of the dead space or of the actual percentage composition of the alveolar air occur during the experiment. The method is described below. For the present we will assume that the actual dead space and the percentage composition of the alveolar air are constant under the conditions of the experiment, and inquire whether or not a deep expiration of normal time and following a normal inspiration contains alveolar air of the same composition or a greater or less dead space than the normal expiration. That the quantities of dead-space air in the two expirations are alike is axiomatic, providing the expirations are large enough to expel completely all of the air which has not taken part in the gaseous exchange of the lung. The alveolar air in the larger expiration will not, however, be exactly equal to that from the small expiration for at least two reasons, i.e., because (*a*) or (1), the diffusion of gases requires an appreciable time, and (*b*) or (2), in the case of the larger expiration the CO_2 excreted or the O_2 absorbed is added to or taken from, respectively, a decreasing volume of alveolar air. This increases the percentage of CO_2 and decreases the percentage of O_2 relatively more than if the same amount of CO_2 was added or an equal amount of O_2 was taken from a large volume of alveolar air. These two factors tend to oppose each other in the case of the deep expiration and consequently hold the value of the alveolar air in the deep expiration near that in the normal expiration. The errors which they make in the method are certainly of small magnitude.

Since we have shown that the mean tension of the alveolar gases is the same during expiration as during inspiration (hence throughout the respiratory cycle), and that the percentages of the alveolar gases in the large and small expiration differ almost solely because of the diluting factor of the dead space, we must conclude that the binomial formula, if applied to data which are correctly taken, is capable of giving with considerable accuracy the mean gaseous composition of the alveolar air and the capacity of the dead space.

We must now inquire into the changes in O_2 pressure which occur during the respiratory cycle. The relationship between the total CO_2 given off and the O_2 absorbed during any period is expressed as a ratio called the respiratory quotient. There is no valid reason for believing that the respiratory quotient obtained from the analysis of one normal expiration should differ from that obtained from a sample of air obtained from ten or fifty respirations at the same time and under like

physiological conditions. But if we take samples of air from different portions of an expiration, the respiratory quotient invariably differs from that obtained from the analysis of a number of normal breaths; and it seems improbable that the discrepancy can be due to experimental error in working with smaller volumes of gas. This has been the universal experience of workers who have estimated the respiratory quotient in alveolar-air samples taken by the Haldane-Priestley method. The explanation for this discrepancy lies in the fact that while the influence of the diluting effect of the tidal alveolar air is marked in altering the amount of CO_2 which is given off by the blood during different phases of the respiration, it has little influence on the amount of O_2 which is taken up by the blood under normal conditions. That this is the case can be deduced from a study of the dissociation curve of the oxyhaemoglobin. At O_2 pressures above 55 mm. the haemoglobin leaves the lung holding practically as much O_2 as at higher pressures. Since the pressure of O_2 under all normal conditions is present in greater degree than 65 mm. in the alveolar air, we cannot expect that, under the same conditions of the circulation and body metabolism, the rate of absorption of O_2 is greatly changed during different times of the respiratory cycle.

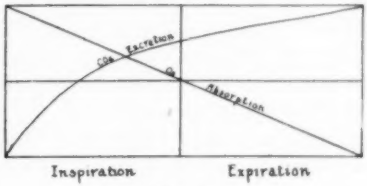

Fig. 3

Krogh (7) and Krogh and Lindhard (8) have attempted by means of diagrams to show the approximate changes in the alveolar CO_2 during inspiration and expiration. They fail, however, to take into consideration the effect of the tidal air in relation to inspiration and expiration (Skand. Arch., xxx, 389, 1913). We have attempted to show diagrammatically these relationships of CO_2 output to O_2 intake in figure 3. The O_2 line is straight, showing that disappearance of cubic centimeters of O_2 from the lung air is practically uninfluenced by the respiratory phases, whereas the CO_2 output is very rapid in the inspiratory and slow during the expiratory phase.

While it is very true that the relationship of the alveolar gases is continually changing throughout the respiratory cycle, the mean relationship of these gases for the period is more or less constant because this is controlled by the type of metabolism. This relationship is expressed by the respiratory quotient. It follows therefore that the ratio of the CO_2 excreted to the O_2 left in the lungs after absorption of

O_2 is, under the same physiological conditions, a constant for any particular period. The average mean level of the alveolar gases must have therefore the same respiratory quotient as that obtained by analysis of samples of the expired air taken from the mixture of several breaths or from one breath of *absolutely average depth and length.*

If the average concentration of the alveolar gases given off during a normal expiration is determined, the respiratory quotient calculated from such data would be the same as that obtained by the analysis of the expired air. Such a calculation, however, would give no information concerning the pressure changes taking place in the composition of the respired gases during any particular time of the respiration.

By Haldane and Priestley's method the estimation of the percentage composition of the alveolar air is obtained from samples of air collected from the end of forced expirations following a normal inspiration and expiration. The average of these values obtained is taken as giving the average gaseous composition of the alveolar air during the respiratory cycle.[1] *Such a conclusion is unwarranted for several reasons.* In the first place, as shown above, the tension of CO_2 in the alveolar air is influenced by the relative amount of CO_2 which is added to the air of the lungs during inspiration and expiration. Since the greater portion is given off at tensions below the average alveolar pressure and during inspiration when the inspiratory and expiratory phases are equal in length, it follows that the tension of the CO_2 is higher than the lowest calculated pressure potentially present in the alveolar air during the respiratory cycle. Moreover, since the evolution of gas is more rapid and greater in amount at low pressures than at high pressures of CO_2, the mean pressure of CO_2 in the alveolar air is reached sometime before the mid-time of expiration, and after more than one-half of the CO_2 given off by the blood during expiration is evolved. Accordingly, an average of the correctly obtained high and low pressures would fail to give the mean tension for CO_2.

Krogh and Lindhard have also called attention to the error present in the Haldane-Priestley method due to the fact that CO_2 is accumulating and O_2 is being absorbed during the time taken for the collection of the samples. This causes the average tension of CO_2 to be calculated as higher and that of O_2 as lower than it actually is. The error

[1] There are several methods for obtaining alveolar air which are based upon the principle of the Haldane and Priestley method. One of these the Fridericia method has been used extensively of late, but it is less accurate in technique and is open to the same criticism as to principle as the Haldane-Priestley method.

cited by Krogh and Lindhard, together with that we have called attention to in the Haldane-Priestley method, accounts for the failure of this method, as mentioned above, to give the same respiratory quotient for the alveolar air as that obtained by the analysis of the expired air of the period.

By taking automatically timed samples of the expired alveolar air from a deep expiration or from several expirations, Krogh and Lindhard (8) attempt to correct the time error and also possibly the error due to the fact that the evolution of CO_2 is faster at low pressure than at high pressures of CO_2. From these data they calculate the probable low pressure of the gas, and from the general average of the whole series of determinations they determine the mean pressure of the CO_2 in the expired alveolar air. The figure thus obtained they find gives a value for CO_2 a little lower, and for O_2 a little higher, than they calculate the alveolar gaseous composition to be from the application of Bohr's formula for the determination of the composition of the alveolar air when the percentage composition of the expired air, the depth of the expiration, and the capacity of the dead space are known. The theory of their idea is correct but the practical application is impossible; because first, samples taken during the course of an expiration, unless they are fortuitously timed, must fail to give an average of the percentage of CO_2 or of O_2 in the alveolar air; and secondly, it is impossible from such curves to determine the composition of the alveolar air at the beginning of expiration, as can be deduced from the points brought out in the beginning of this paper.

In the data which Krogh and Lindhard present, however, we fail to understand how they got such widely varying percentages of O_2 in the inspired air. The highest value for O_2 in the atmospheric air as determined from two hundred and sixty-four analyses of outside air on Pike's peak, the ocean, in England and Boston, is 20.938 per cent and the lowest 20.927 per cent (Benedict, Carnegie Institute Reports, 116). We have confirmed this in a large number of determinations made by us over a considerable period for Cleveland air. Such variation in the percentage of O_2 in the inspired air as given by these authors—unless inspired air was made up of artificial mixtures of O_2 with some inert gas, as is not stated—seems to us quite impossible, and throws grave doubt on the accuracy of the O_2 figures.

Bearing the above factors in mind, we have sought for a method for the determination of the average composition of the alveolar air which would be free from the fault inherent in the other methods. Such a

method was presented by me some months ago in the first paper of this series. The details of the method were at that time not so finished as I hoped to make them; hence detailed directions were not given but were reserved for the present paper. The method depends on the relationship existing between the diluting influence of the dead space and the percentage composition of the alveolar-air gases in expirations of varying depths, but of *normal time* and following *normal inspirations*. Professor Almeida had published the application of this mathematical principle as applied to alveolar-air determinations in a preliminary communication in the Brazil Medico for June 27, 1916, announcing the general principle involved. This had been used in my laboratory some time previous to the appearance of Almeida's article, and it was not until after we had accumulated the data used in the first paper of the series that our attention was called to his article. The formula was used by us in an attempt to determine the true capacity of the air passages, and later for the determination of the composition of the alveolar air. It is impossible simply to make a small expiration into one spirometer and a large one into another, and from the analysis of these to deduce the percentage of CO_2 or of O_2 in the alveolar air, as is suggested by Almeida. The greatest care must be taken that the respiratory rate be controlled solely by the respiratory center; that the inspiration preceding the taking of each sample be exactly one of average normal depth and length; that the expiration include all the expired air; that, be it large or small, it take the time of the normal expiration; and lastly, what is perhaps most significant of all, that the respiratory quotient be the same in the large expiration as in the small. Unless all these factors be cared for the greatest variation must occur, since the same factors which produce error in Haldane and Priestley's method will enter in.

Methods. In order to remove these possible sources of error, we have sought to produce an apparatus which will be essentially automatic and which will show graphically any deviation from the accepted standard occurring in collecting the expired air. Such an apparatus must include an efficient valvular mechanism to partition the incoming and outgoing air, working with a minimum resistance, and connected with a mouth or nose piece containing a minimum dead space; accurate spirometers to measure the expired air; a timing mechanism which will indicate the exact time taken to collect the total large expiration, and the average time taken for each respiratory cycle in the series of respirations, from which the normal expiration is obtained by average; a

pneumograph of sufficient delicacy to detect any abnormality in the character of the inspirations; and a Haldane gas apparatus for analyzing the samples obtained.

Valves and nose and mouth piece. A great deal of difficulty was experienced in finding a method to partition accurately the inspired and the expired air. Since it is absolutely essential for the success of the method that all the dead-space air be obtained and that only the normal pause be made between inspiration and expiration, the valves must approach 100 per cent efficiency. Carpenter reports that the only valves approaching this are Tissot valves. It is impossible to obtain these at present, and besides they have certain objections which make them eminently unsuited to the work at hand. The Zuntz valves contain too large a dead space.

Working with a view to meet these requirements, we have finally produced a valve which is very simple and very easily made. The prepared casings used for the manufacture of bologna are obtained from a sausage maker. These are preserved in salt and will keep indefinitely if placed on ice. When needed a short bit is taken, washed free from salt by allowing water from the tap to run through it, and placed in a weak glycerin solution. The gut becomes very soft and pliable and does not dry quickly. A piece of bologna casing about 10 cm. long is threaded through a glass tube of about 15 mm. bore and 4 to 6 cm. in length. One end of the casing is brought around the outside of the tubing and secured by means of a thread. The lower end of the membrane is pinched off and the casing is distended by blowing through the free end of the tube. The casing is then cut a little more than half way across its middle, so that this opening will lie just within the free end of the tube when the casing is drawn back through the tube. The loose end of the casing is given a *little twist, an essential procedure determined by experience,* and this end is secured by a thread on the outer side of the tube. If properly made, the valve should work freely without vibration and the opening be sufficiently large to allow a good current of air to pass. It should collapse instantly and be air-tight when the current of air is reversed. The back lash, or lag of closure, of these valves is extremely small and they will open or close with a pressure of air little exceeding the pressure changes of air in normal respiration. When not in use the valves should be kept in glycerin water on ice. Valves prepared in this way have been in use a month without loss of efficiency. They are, however, made with so great ease that new valves are provided for each subject, and they are therefore especially adapted

toward work. These valves are inserted in reverse order into a supporting metal T-piece, and the joints made air-tight by tape. The stem of the T is conected with a nose or mouth piece. The mouth piece recommended is the Siebe-Gorman mouth piece used in mine-rescue work, and a Siebe-Gorman nose clip is used. If breathing through the mouth is uncomfortable with the nose stopped, we have found it advisable to use nose breathing. For this we have used a nasal mask made of tin and fitted air-tight on the face with rubber adhesive tape. The mask and valves have a dead space of approximately 15 to 20 cc. if made correctly. (The valves are illustrated in figure 4.)

The spirometers. The expired air is collected in spirometers essentially the same as the well known Krogh type. The spirometer for the single expiration is of a capacity of 1500 cc. and is capable of being read with an accuracy of 5 cc. The larger spirometer, in which a number of breaths are collected, holds 5500 cc. and can be read with an accuracy of 15 to 20 cc. The small spirometer is made to record the exact time

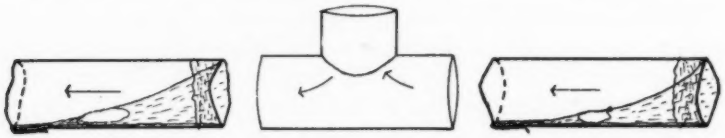

Fig. 4

during which air enters the spirometer by means of a grooved dial on the axis of the lid, on which works a thread over a system of pulleys, to which is attached a writing point so that any movement of the spirometer may be synchronously and accurately recorded on the smoked paper. A three-way cock is in the air way leading to each spirometer. The spirometers are closely connected and arranged so that the air current may be directed in the three following ways: (1) through cocks *1* and *2* outside; (2) directly through both valves into the large spirometer for the purpose of collecting a series of expirations; and (3) through cock *1* directly into the small spirometer for catching single expirations. In all experiments the first filling of the spirometer is rejected, so that the dead space of the spirometers is filled with air of approximately the same composition as in the succeeding expirations. The time is marked in seconds by a Porter time clock. The respiratory movements are recorded by a pneumograph. The arrangement of the apparatus is shown in figure 5.

Experimental procedure. The subject is brought into respiratory equilibrium by having him breathe through the valves for a period of time before the observation period. The respiratory movements during this time are recorded while the cocks are in *position 1*. When the observation is started, the cocks are turned into *position 2* during the time an inspiration is being made, so that the following expiration may be collected in the large spirometer. After about ten respirations (a counted number), the cocks are turned to *position 3* during an inspiration, so that a single expiration may be collected in the small spirometer. This expiration must be deepened and hence must be commensurately quickened in order to keep the time of expiration normal. This is more or less a chance procedure but a trained subject can approximate

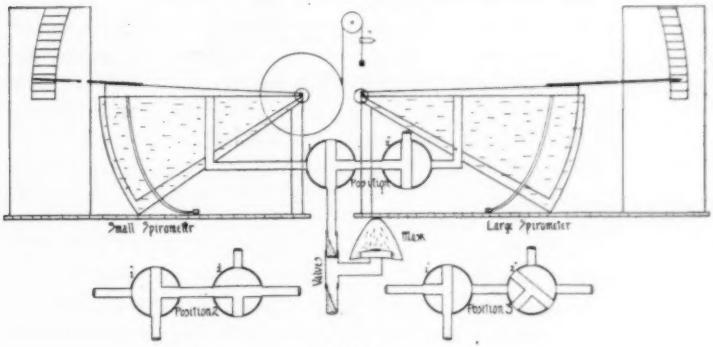

Fig. 5

the condition with considerable accuracy. Any gross variation from the normal time is sufficient reason to discard the observation. Often the inspiration immediately preceding the expiration into the small spirometer is varied involuntarily by the subject on account of his being aware that the following expiration must be deepened and quickened. For these reasons it is almost impossible to make check experiments, and averages must be resorted to. A criterion for accuracy is the respiratory quotient, as is shown in the first part of this paper. Any variation from the normal in any way will affect the respiratory quotient of the deep expiration; therefore the quotients from the small and the large expirations must approximately check. The gaseous composition of the air is determined by the Haldane method. We have found a 30 cc. ground-glass aspirating-syringe equipped with a two-way stop-

cock very convenient for obtaining samples for analysis. We find these much quicker and less troublesome than the Haldane tubes, as they do away with the necessity of evacuation or the use of mercury. They are perfectly reliable if the pistons are well greased and care is taken to wash out their dead space with the analyzed air before drawing off the sample. By working the piston of the syringe back and forth a number of times, the air is thoroughly mixed in the spirometer before a sample is withdrawn.

A protocol of an actual experiment is given in the first part of this paper. From the data thus accumulated may be calculated the volume of the dead space for both O_2 and CO_2, and the average tension of the CO_2 and O_2 in the alveolar air during respiration, by the formulas as given on page 377 of this paper. If the technique is good, the respiratory quotient of the alveolar air as thus determined is approximately that of the average expired air. Since this method is in accord with the theoretical criteria for accuracy, we believe that the values obtained by it must be fairly near the true figures. The result obtained at the same time for the composition of alveolar air by the Haldane-Priestley method are from 6 to 15 per cent higher for CO_2, and from 12 to 20 per cent greater for O_2 absorbed than those obtained by this method during conditions of *rest*. This is in accord with the fact that the respiratory quotient of alveolar air samples obtained by the Haldane-Priestley method are usually much lower than those obtained from the analysis of the total expired air. The following table is compiled from data obtained on R. G. P. in experiments under a condition of rest. The variation occurring in the percentage of CO_2 in the alveolar air during the day is interesting. We have noted this variation in many observations and are certain it is not due to experimental error. The subject is being investigated in our laboratory at present. The change made in the calculation of the dead space and the alveolar air by the modification of the respiration is also of interest with especial regard to the relative dead spaces of CO_2 and O_2. This point will be taken up in the following paper.

It has been practically impossible to use the method as described on patients. The use of the kymograph and pneumograph, together with the other complicating factors, makes it quite impracticable as a clinical procedure. We have, however, used a slight modification of the method with fair results.

In this case after the patient has become accustomed to breathing through the valves, we catch a single normal expiration in the small

TABLE 2

AMOUNT	EXPIRED AIR				ALVEOLAR AIR				DEAD SPACE		REMARKS
	CO_2	O_2	O_2 absorbed	Respiratory quotient	CO_2	O_2	O_2 absorbed	Respiratory quotient	CO_2	O_2	
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*cc.*	*cc.*	
885	4.45	15.55	5.65	0.780							April 17, 1917
470	3.82	16.20	4.92	0.777	5.15	14.75	6.48	0.794	124	113	9 a.m.
825	4.10	15.98	5.17	0.791							
518	3.65	16.40	4.67	0.783	4.76	15.02	6.23	0.764	128	116	11 a.m.
700	4.26	15.45	5.80	0.720							
462	3.90	15.85	5.43	0.740	4.97	14.68	6.61	0.752	102	78	Noon
890	4.49	15.51	5.69	0.789							
500	3.94	16.02	5.15	0.766	5.26	14.84	6.32	0.832	119	97.5	2 p.m.
1335	4.93	14.94	6.26	0.788							
510	4.10	15.95	5.37	0.787	5.47	14.36	7.09	0.780	126	124	3 p.m.
915	4.85	14.70	6.73	0.720							Long expiration prolonged by one-half normal time
510	4.07	15.85	5.37	0.758	5.87	13.33	8.08	0.726	154	186	3 p.m.
1260	5.90	14.95	6.29	0.779							Respirations were quickened 20 per cent for 40 seconds
480	4.04	15.70	5.47	0.739	5.35	16.10	5.24	1.020	87	318	

spirometer by turning the stopcock from position *1* to *3* (fig. *3*). We then take a sample of this for analysis. In succeeding samples we request the patient after he has started to expire into the spirometer following a normal inspiration to deepen the expiration. By this means we can collect several expirations which differ from each other in increasing amounts. The percentage of CO_2 is plotted on cross-section paper on the ordinates, with the cubic centimeter in the expiration on the abscissae. A line joining these should make a hyperbolic curve. Any marked deviation from such a curve indicates at once that an error has been made in taking the sample, and this sample should be discarded. The determination of the CO_2 percentage of expired air is so simple that a number of specimens of varying depths of expiration can be taken and thus many points on the curve determined. For the most accurate results it is best in general to compare only those expirations which differ from each other by at least 0.3 per cent in CO_2 and by at least 200 cc. in volume. That this is best is shown, as suggested by Almeida, from the fact that the diluting effect of the dead space in

reducing the percentage of CO_2 in the expired air from the percentage of CO_2 in the alveolar air is greater in relatively small expirations. If more exact work is wished, the O_2 content can be determined on each specimen, the respiratory quotient calculated, and only those expirations which show the same respiratory quotient combined. The following tables give data obtained on two occasions from observations made on R. G. P. by the above method.

TABLE 3

Wednesday morning

NUMBER OF OBSERVATION	TOTAL EXPIRED AIR	CO_2 IN EXPIRED AIR	ALVEOLAR CO_2			DEAD SPACE		
			1	2	3	1	2	3
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*
1	450	3.10						
2	637	3.66	4.99			170		
3	750	4.00	5.34			189		
4	960	4.28	5.30	5.48	5.27	189	183	214
5	1120	4.30	5.11	5.15	4.92	161	140	184
6	1440	4.40	5.16	4.98	4.82	171	127	171

General average for CO_2 in alveolar air, 5.13 per cent.

General average for dead space, 172 cc. Dead space in valves in this experiment was about 20 cc.

The Haldane-Priestley method gave 5.60 per cent as the mean alveolar CO_2 pressure on the occasion of this experiment.

TABLE 4

Wednesday afternoon

NUMBER OF OBSERVATION	TOTAL EXPIRED AIR	CO_2 IN EXPIRED AIR	ALVEOLAR CO_2				DEAD SPACE			
			1	2	3	4	1	2	3	4
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*
1	430	3.60								
2	655	4.55	6.41				187			
3	795	4.75	6.13				175			
4	840	4.85	6.15	5.90			179	151		
5	1230	5.05	5.82	5.60	5.56	5.47	164	124	120	96

Average CO_2 5.88 per cent. Dead space 149 cc. Dead space of valves about 20 cc.

The Haldane-Priestley method gave 6.20 per cent for CO_2 in alveolar air.

The fact that the increase in the percentage of CO_2 found in expirations of increasing depths is directly related to a constant dead space

is shown by the relatively small variations found in the percentages of CO_2 obtained by the various combinations. Incidentally for like reason we must believe that the mixture of gases in the alveolar air is quite uniform at the end of an inspiration and not incomplete as is claimed by Sonne (9).

SUMMARY

The blood in the pulmonary capillaries contains less CO_2 during inspiration than during expiration. The alveolar air receives a greater amount of CO_2 during inspiration than during expiration.

The greater amount of the CO_2 given off during the respiration cycle occurs at a pressure less than the mean; the less amount is given off at a pressure above mean.

The alveolar CO_2 tension rises relatively more rapidly during the termination of inspiration and during the first part of expiration than during the latter part of expiration. This condition is due to the fact that during the first part of expiration the pressure of CO_2 is less than in the latter part, inasmuch as the total volume of air is reduced by the expiratory act, and each added increment of CO_2 is added to progressively decreasing amounts of air, and accordingly the percentage of CO_2 mounts very rapidly; even though the actual evolution of CO_2 from the blood is less at high partial pressures of CO_2 than at low pressures.

The rate of O_2 intake of the blood is practically constant during both phases of the respiration at ordinary barometric pressures.

A method based upon theoretical grounds is proposed for the determination of the gaseous composition of the alveolar air and for the capacity of the dead space for both O_2 and CO_2.

We believe that the results obtained by this method give very accurate values for mean tensions of CO_2 and O_2 in the alveolar air. Samples obtained by the Haldane-Priestley method give figures 6 to 15 per cent higher for CO_2, and 9 to 20 per cent higher for O_2 *absorbed*, than are obtained by this method. The dead space determined by this method fails to show as great variation as when determined by the Haldane-Priestley method, and the dead spaces for O_2 and CO_2 are of the same magnitude within the experimental error.

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STUDIES IN THE PHYSIOLOGY OF THE RESPIRATION

III. The Oxygen and the Carbon Dioxide Dead Space in Man

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This problem has been the subject of much debate in recent years. Krogh and Lindhard champion the idea that the dead space is a more or less unchangeable anatomical affair and that the composition of the alveolar air can be deduced from calculations based upon the percentage composition of the expired air, the volume of the respiration and the capacity of the dead space. With this the school of Haldane disagrees. Haldane and Priestley estimate the capacity of the dead space by obtaining directly a sample of the alveolar air, the total expired air and the percentage gaseous composition of each. They combine them in a formula as follows:

$$\text{Volume of expiration} - \frac{\text{Per cent } CO_2 \text{ expired air} \times \text{cc. in expired air}}{\text{Per cent } CO_2 \text{ alveolar air}} = \text{Dead-space air.}$$

The factor of this equation the determination of which is open to criticism since it is obtained by a more or less empirical method, is the percentage CO_2 in the alveolar air. The volume of expiration and the CO_2 in the expiration are subject to absolute experimental estimation, and if any error is present it must be in the determination of the composition of the alveolar air, and it is upon this point that, with the aid of the binomial formula for the determination of the dead space, we intend to approach the problem. The soundness of this formula has been fully discussed in previous papers. If such a mathematical formula, theoretically sound and utilizing absolutely normal data, fails to give correct values for the composition of alveolar air or for the dead space, we are at a loss to find a basis to solve the problem. Using the Haldane-Priestley method for the determination of the CO_2 in

the alveolar air, Douglas and Haldane (1) found that the dead space increased greatly during the deepened breathing resulting from various grades of exercise.

The results of Haldane and Douglas were severely criticised by Krogh and Lindhard (2), who called attention to an error inherent in the method of obtaining samples of alveolar air by the Haldane-Priestley method. This error they point out is sufficient to produce the results obtained by Douglas and Haldane in the deep breathing of exercise. However, the criticism of Krogh and Lindhard was not accepted by the Haldane school and in a later paper Haldane expresses agreement with the views presented by Henderson, Chillingworth and Whitney (4), that deep breathing alone causes the dead space to be greatly increased, and furthermore he advances the theory that the dead space for O_2 is greater than that for CO_2.

Very recently Krogh and Lindhard (5) have again opened up the subject. In a new series of experiments they have carefully gone over the whole question and fail to confirm the work of Haldane or Henderson. They do find, however, that there is an increase of perhaps up to 100 cc. in the dead space when very deep breaths are taken and that under all normal conditions of breathing the dead space varies very little. Henderson and Morriss (6), in a paper just at hand, interpret these results as confirming the experiments of Haldane and of Henderson, Chillingworth and Whitney, in which there is found an increase of from 400 to 600 per cent in the dead space during deep breathing. In doing this they are in error, having failed to understand the spirit of Krogh and Lindhard's criticism.

In the first paper of this series (7), which appeared at the same time as the last paper of Krogh and Lindhard, one of us (R. G. P.) proposed a new method for the estimation of the dead space, the details of which are given in the second paper of the series. In this paper we pointed out that the dead space did not appreciably vary (30 to 40 cc., which is probably within range of the experimental error of the method) in the deep breathing which occurs during exercise of a degree closely approaching that in which Haldane and Douglas found an increase of nearly 300 per cent. Moreover, the very data from which Douglas and Haldane computed their results, when recalculated by the binomial equation proposed, gave results which showed conclusively that during the various degrees of depth of breathing there was little variation and none that was constant either in the dead space or in the percentage of CO_2 in the alveolar air. A curve was also drawn representing the dilu-

tion curve of the alveolar air by dead-space air from the average figures obtained by the recalculation of the data given by these authors. The points fell very close to the average in every case. The theory of probabilities prevents us from putting any other interpretation on such a result than that the dead space and the composition of the alveolar air remain practically constant or at least within the range of experimental error, during the variations in the depths of breathing encountered. These results support the claim of Krogh and Lindhard that the percentage composition of the alveolar air can be deduced accurately under ordinary conditions by the analysis of a sample of the total expired air if one has a knowledge of the dead space. The papers of Krogh and Lindhard and of Pearce show beyond question that the use of the Haldane-Priestley method for the estimation of the dead space is impossible. The error present in the method has been gone into in detail in the second paper of this series.

In the present paper we wish to present data confirming our view that the dead space is a relatively fixed anatomical and physiological entity, and also to show that the dead spaces for CO_2 and O_2 are essentially the same under all conditions in which the breathing is in accord with the physiological needs of the body, and that the variations which Haldane and Henderson and their coworkers find are artifacts and due to faulty methods and to artificial modes of breathing.

Haldane gives data which he obtained by increasing the depth of the respirations voluntarily for three minutes while the rate of breathing was under the control of the respiratory center. If the minute volume were controlled by the respiratory center, the CO_2 excreted per unit of time should remain normal at any rate and depth of breathing. In this case the data supply all the requirements for the calculation of the dead space and the percentage composition of the alveolar air by the binomial formula. We have accordingly recalculated the figures which Haldane gives for the alveolar gases and the capacity of the dead space, and fail to confirm his results. The figures obtained are very irregular but this is to be expected, since Haldane apparently was unable to comply with the conditions of the experiment, as is attested by the facts that the total CO_2 excreted per minute was not the same in all cases and that the respiratory quotient was not constant during the experiment.

In the following tables we have made all possible combinations in the binomial formula of Haldane's data (3) as is indicated by the figures showing the number of the observations (table 5). Table 1 gives the

TABLE 1

Alveolar CO_2

	1	2	3	4	5	6	7
2	3.12						
3	4.27	5.00					
4	4.64	5.13	4.49				
5	4.67	4.94	4.80				
6	4.40	4.92	4.84				
7	4.47	4.77	4.64	3.44	5.10	4.03	
Average..	4.26	4.65	4.67	4.42	4.88	4.55	4.41

Average obtained by combining 2 with 4, 5 and 6 (expirations which have the same respiratory quotient) is 5 per cent.

TABLE 2

Alveolar O_2

	1	2	3	4	5	6	7
2	16.85						
3	15.7	15.50					
4	15.7	15.41	15.8				
5	15.8	15.65	16.0				
6	15.8	15.53	16.1				
7	15.9	16.03	16.2	17.4	16.5	16.97	
Average..	15.78	15.62	15.88	16.08	15.98	16.02	16.45

Average obtained by combining 2 with 4, 5 and 6 (samples having the same respiratory quotient) is 15.52 per cent.

TABLE 3

Dead space CO_2

	1	2	3	4	5	6	7
2	−117						
3	315	210					
4	212	234	241				
5	130	176	163	268			
6	131	174	161	297	417		
7	114	136	127	266	97	98	
Average..	131	155	203	253	209	213	140

Average obtained by combining 2 with 4, 5 and 6 (expirations which have the same respiratory quotient) is 194 cc.

values of CO_2 in the alveolar air; table 2 those for aveolar O_2; table 3 those for CO_2 dead space; table 4 those for O_2 dead space; while table 5 gives the general average obtained from a comparison with Haldane's results.

TABLE 4

Dead space O_2

	1	2	3	4	5	6
2	−394					
3	245	353				
4	207	230	191			
5	167	181	131	222		
6	150	173	112	134		
7	99	110	71	50	213	311
Average..	165	209	150	172	182	176

Average obtained when 2, 4, 5 and 6 are combined is 194 cc.

TABLE 5

NUMBER OF OBSERVATION	RESPIRATORY RATE PER MINUTE	DEPTH OF EXPIRATION AT 37° SATURATED	EXPIRED AIR			ALVEOLAR CO_2 PER CENT		ALVEOLAR O_2 PER CENT		DEAD SPACE			
			CO_2 per cent	O_2 per cent	Respiratory quotient	Haldane's	Ours	Haldane's	Ours	Haldane's CO_2	Ours	Haldane's O_2	Ours
		cc.								cc.	cc.	cc.	cc.
1	3.0	2984	4.29	16.07	0.848	5.72	4.26	13.70	16.23	683	131	920	165
2	4.0	2438	4.56	15.91*	0.875	5.66	4.65	14.02	15.83	467	155	619	209
3	6.0	1413	4.24	16.47	0.905	5.53	4.67	14.38	15.88	272	202.8	392	150
4	18.5	683	3.31	17.27	0.871	5.64	4.42		16.08	224	253		172
5	17.0	650	3.59	17.01	0.887	5.71	4.88	13.84	15.98	171	209	223	182
6	17.7	643	3.58	16.98	0.888	5.55	4.55	13.72	16.02	161	213	231	176
7	24.0	410	3.22	17.15	0.780	5.45	4.41	13.65	16.45	111	140	136	175

Average when 2 is compared with 4, 5 and 6 (expirations which have nearly the same respiratory quotient):

ALVEOLAR		DEAD SPACE	
CO_2	O_2	CO_2	O_2
5.00	15.53	194	194

We wish to call attention to the fact that those observations which have nearly the same respiratory quotient give results which vary at most from their mean for CO_2 by 0.13 per cent; for oxygen by 0.11 per cent; for CO_2 dead space by 40 cc.; and oxygen dead space by 36 cc.

* Evidently misprinted in Haldane's table, where it reads 16.91.

Haldane finds that the respiratory quotient obtained from the alveolar air sample is much lower than that obtained in the expired air, and since the composition of the alveolar air is a basic figure in the computation of the dead space by the Haldane-Priestley method, an increase in the percentage of O_2 absorbed will give a larger dead space for O_2 unless the CO_2 excreted is increased in the same proportion. As pointed out in the preceding paper, the respiratory quotient of the average alveolar air must be the same as that of the average tidal air; otherwise the subject would eventually blow up or vanish into air.

An analysis of the physical and mathematical laws governing gas exchange in the lungs will at once show the location of Haldane's and Henderson's error. During expiration, as shown in paper II of this series, the CO_2 comes off from the blood with progressively less rapidity but into a decreasing amount of alveolar air. Each added increment of CO_2 therefore increases the percentage of CO_2 in the alveolar air disproportionately at any time during the expiratory phase. What is true of CO_2 is true of O_2 in the reverse way, save in this respect that, while the rate at which CO_2 is evolved is decreased by each increase in the amount of CO_2 in the lung air due to the rapidly increasing tension of the gas, with O_2, where the tension under all normal conditions is much more than sufficient to saturate the haemoglobin completely, the rate of absorption is practically unaffected by the decreasing tension of O_2, which must occur rapidly as each cubic centimeter absorbed from the blood is taken from a less and less volume of alveolar air. This must result in a low respiratory quotient when the sample of air is taken from the end of a deep expiration, be it rapid or slow. In going over the published figures of Haldane, and in general the published figures given for alveolar respiratory quotients as estimated by the Haldane-Priestley method, they are found to be almost invariably lower than those obtained by the analysis of the tidal air.

Besides this factor there enters the time factor error, pointed out by Krogh and Lindhard. There can be no doubt that the Haldane-Priestley method gives too high results for CO_2 and too low results for O_2 alveolar pressure, and that the percentage of O_2 absorbed as estimated by the method is more than proportionately greater than the percentage of CO_2 excreted. That this is the case is seen by the recalculation of the tables of Haldane. It is seen that there is no progressive increase in the dead space or in the alveolar air CO_2 or in the O_2 absorbed in this series. If there was a progressively increasing dead space as the depth of respiration increased, then there should be a

progressively changing value for the CO_2 and O_2 tensions found in the values when each expiration was combined with all the others of the series, and likewise the dead space value for both O_2 and CO_2 should show a progressive change. No such change is noted in the tables. We have tried time and again to modify our respirations voluntarily after the method proposed by Haldane but have not been any more successful than he was in keeping the respiratory quotient constant. If his experiments were all done on the same day and at the same period his results show a wide variation in the value of the respiratory quotient and indicate that he was not ventilating his blood according to the needs of his body or controlling his respiration by his respiratory center. Such being the case, the relatively small variations which we find by recalculating his results are perfectly clear.

We have attempted to devise experiments that will give results simulating those of Haldane and from these to demonstrate experimentally Haldane's error. The outline of the experiments is as follows.

The first experiment was to determine the effect of deep breathing on the dead space: *a*, by increasing the dead space, and *b*, by voluntarily increasing the depth of respiration and keeping the alveolar tidal minute volume normal.

It is well known that any artificial increase in the dead space, such as is present when breathing is accomplished through a tube, will increase the volume of the respiration. If deep breathing increases the capacity of the dead space we should find that the dead space calculated by the binomial formula under normal conditions will increase more than by the amount of the added dead space during the deepened breathing through the tube. The reason for adding the artificial dead space is simply to obtain an augmented respiration which is apparently controlled by the respiratory center.[1] We arranged a very simple experiment in which the volume of the connection between the nose-mask and the valves was increased by 50 and then by 100 cc., respectively, and the volume of the dead space was determined for CO_2 when the subject was breathing through nose-mask and valves, then when he breathed through the same but with 50 cc. added dead space, and then with 100 cc. added dead space. Table 6 gives the results obtained in such an experiment. It is seen that the anatomical dead space remained unchanged with the deepened breathing which the addition of artificial dead space produced.

[1] We are indebted to Dr. Thorne M. Carpenter for this suggestion.

Since there is much difficulty in increasing the depth of breathing and keeping it within the requirements of the body even with the above method, we have devised an experiment wherein all factors can be actually mathematically controlled.

On this particular occasion the average tidal air was 488 cc.; the time consumed in a single respiratory cycle was 4.5 seconds; the dead space, as calculated by the binomial formula, was 103 cc. for CO_2 and 116 for O_2. Subtracting 103 from 488, we have 385 cc. for the alveolar tidal air. Now, we desired to double the depth of inspired alveolar air

TABLE 6

AMOUNT	EXPIRED AIR				ALVEOLAR AIR				DEAD SPACE		REMARKS
	CO_2	O_2	O_2 absorbed	Respiratory quotient	CO_2	O_2	O_2 absorbed	Respiratory quotient	CO_2	O_2	
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*cc.*	*cc.*	
906	4.63	15.43	5.750	0.805							Normal control. July 28, 9.30 a.m.
601	4.20	15.90	5.270	0.810	5.39	14.28	7.01	0.780	140	128	
970	4.40	15.78	5.370	0.819							Dead space increased 50 cc. July 28, 9.30 a.m.
775	4.11	16.10	5.240	0.815	5.44	14.36	6.89	0.789	198	192	
1065	4.75	15.90	5.175	0.926							Normal control. 3.30 p.m., July 31.
542	3.97	16.71	4.280	0.927	5.60	14.95	6.10	0.917	155	155	
1320	4.35	16.30	4.730	0.920							Dead space increased by 100 cc. 3.30 p.m. July 31
845	3.73	16.94	4.080	0.915	5.47	15.15	5.89	0.929	267	259	

but at the same time to keep the minute volume of the alveolar tidal air constant. In order to accomplish this, we inspired from a Krogh spirometer two, three or four times the volume of the alveolar air plus the volume of the calculated dead space, and expired the same volume, the whole process occupying approximately two, three or four times, respectively, the time of a normal respiration; the inspiratory and expiratory phases being kept approximately equal. The subject timed himself by a stop-watch, so that he soon learned to inspire and expire the proper amount in the correct time. In order to insure accuracy,

the movements of the spirometer were recorded and timed, as is shown in the tracings (fig. 1).

Table 7 gives the results of this experiment. It will be noted that the alveolar CO_2 and O_2 remain practically unchanged and that the volumes of the dead space for O_2 and CO_2 are alike and do not change with the increasing depth of inspiration. The variations which do occur are seen to correspond with the variations in the respiratory quo-

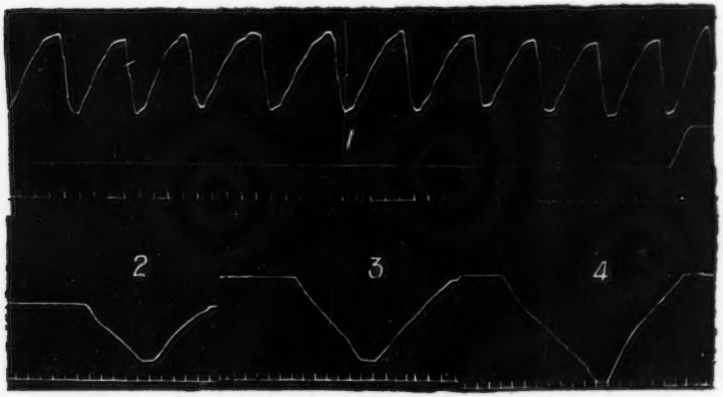

Fig. 1. Tracing 1 is that obtained in the determination of the percentage composition of the alveolar air, the capacity of the dead space and the length of the normal respiratory cycle. Tracing 2 is that obtained when the alveolar tidal air and the length of the respiratory cycle were increased approximately two times normal. Tracing 3 is that obtained when the alveolar tidal air and the length of the respiratory cycle were increased approximately three times normal. Tracing 4 is that obtained when the alveolar tidal air and the length of the respiratory cycle were increased approximately four times normal.

tient. The demonstration of this is seen in the following table (8), in which are given data of experiments, the respiratory quotients of which failed to check. It is quite apparent that the relationship of the dead spaces of CO_2 and O_2 varies with the lack of agreement in the respiratory quotients in the compared observations irrespective of the depth of respiration. In some cases the O_2 dead space becomes apparently greater, in others less, than the CO_2 dead space. The importance of this factor is discussed in paper II of this series.

TABLE 7

	VOLUME OF ALVEOLAR TIDAL AIR	VOLUME OF INSPIRED AIR	VOLUME OF EXPIRED AIR	CO_2	O_2	O_2 ABSORBED	RESPIRATORY QUOTIENT	ALVEOLAR CO_2	ALVEOLAR O_2	ALVEOLAR O_2 ABSORBED	ALVEOLAR RESPIRATORY QUOTIENT	CO_2 DEAD SPACE	O_2 DEAD SPACE	REMARKS
1	385	488	488	4.10	15.73	5.420	0.757							
2	385	488	680	4.41	15.36	5.900	0.748	5.22	14.15	7.22	0.723	103	116	Alveolar tidal air normal. Respiration 4.5 seconds.
3	385	488	488	4.10	15.73	5.416	0.757							Alveolar tidal air 2 times normal.
4	770	873	870	4.51	15.06	6.250	0.721	5.00	14.13	7.30	0.685	104	126	Respiration 10 seconds.
5	385	488	488	4.10	15.73	5.416	0.757							Alveolar tidal air 3 times normal.
6	1155	1258	1264	4.82	15.00	6.250	0.771	5.27	14.56	6.69	0.788	108	98	Respiration 13.5 seconds.
7	385	488	488	4.10	15.73	5.416	0.757							Alveolar tidal air 4 times normal.
8	1540	1643	1650	4.87	14.96	6.285	0.774	5.13	14.62	6.65	0.772	103	91	Respiration 16 seconds.

In order to show experimentally the effect which any alteration in the depth of the inspiration directly preceding the expiration will have on the value of the respiratory quotient, and therefore indirectly on the dead spaces, we made the following two experiments: *a*, in which the inspiration was deepened, the time being kept normal; and *b*, in which the inspiration was decreased, with normal time. The results are:

	AMOUNT OF INSPIRATION	EXPIRATION	CO_2	O_2	R. Q.
	cc.	*cc.*	*per cent*	*per cent*	
a	1080	650	3.10	17.65	0.929
b	300	575	4.43	14.61	0.649

TABLE 8

To show the importance of check respiratory quotients in the binomial formula

AMOUNT	EXPIRED AIR				ALVEOLAR AIR			DEAD SPACE			TIME OF EXPIRATION IN SMALL SPIROMETER
	CO_2	O_2	O_2 absorbed	Respiratory quotients	CO_2	O_2	O_2 absorbed	Respiratory quotients	CO_2	O_2	
(1) 1160	4.60	14.90	6.63	0.695	5.03	13.67	7.87	0.639	914	235	Short
(5) 590	4.22	16.10	5.01	0.842							Short
(1) 1160	4.60	14.90	6.63	0.695							Short
(6) 600	4.15	15.85	5.35	0.805	5.08	13.89	7.58	0.672	110	199	Short
(2) 1110	4.80	15.15	6.07	0.790							Short
(5) 590	4.22	16.10	5.01	0.842	5.47	14.03	7.30	0.748	133	186	Short
(2) 1110	4.80	15.15	6.07	0.790							Short
(6) 600	4.15	15.85	5.35	0.805	5.57	14.27	6.92	0.799	152	136	Short
(3) 110	4.72	15.15	6.08	0.778							Long
(7) 550	4.05	16.35	4.74	0.855	5.43	14.00	7.45	0.737	136	159	Long
(3) 1100	4.72	15.15	6.08	0.778							Long
(8) 585	4.05	16.00	5.19	0.783	5.50	14.25	7.10	0.776	153	157	Long
(4) 1070	4.75	15.20	6.01	0.790							Long
(7) 550	4.05	16.35	4.74	0.855	5.53	14.03	7.29	0.759	145	192	Long
(4) 1070	4.75	15.20	6.01	0.790							Long
(8) 585	4.05	16.00	5.19	0.783	5.50	14.01	7.33	0.750	162	152	Long

At the time the above observations were made, the percentage of CO_2 in the alveolar air was 5.3 per cent and the percentage of O_2 absorbed was 6.5, giving a respiratory quotient for the alveolar air of 0.81. Using the formula of Haldane and Priestley, the dead space for CO_2 in observation *a*, would be 262 cc., and for O_2, 310cc. In observation *b*, however, the dead space for CO_2 would be 107 cc., and that for oxygen 36 cc.

In order to show the importance of the time element in the respiratory quotient and therefore indirectly upon the dead spaces, we did the following experiment: After a normal inspiration we made expirations of approximately the same depth (1575 cc.) but from 1.5 to 11 seconds in length.

	VOLUME	TIME	CO_2	O_2	RESPIRATORY QUOTIENT
		seconds			
a	1600	11	5.86	11.38	0.556
b	1575	3.5	5.43	13.95	0.723
c	1565	1.5	4.95	14.80	0.767

The dead spaces for O_2 and CO_2 in the above experiment as calculated by the Haldane and Priestley formula, the alveolar CO_2 and O_2 being the same as in the preceding experiment, are:

	CO_2	O_2
a	−167	−990
b	−34	−216
c	+105	+65

These experiments show the importance of keeping the amount of the inspired air and the time of respiration constant when the capacity of the dead space is to be calculated from the results of the analysis of the expired air and the percentage composition of the alveolar air. The above experiment was intentionally made extreme, but the error is also extreme. One can hardly conceive of a "virtual" negative dead space. It may be claimed that the error in the above experiments lies in the composition of the tidal air, which is subjected to small experimental error in an actual experiment. This is true, but the change in the tidal air we have demonstrated lies wholly in its alveolar portion and any method which seeks to determine the capacity of the dead space from the percentage composition of the normal alveolar air by direct analysis of a sample of alveolar air will show like results, but with positive dead spaces where we find negative ones, if the alveolar samples are taken from respirations of the type in the above experiments. This also shows that the rate of O_2 intake is subject to less fluctuation due to tension changes occurring in the lung during respiration than the rate of CO_2 output.

SUMMARY

The experiments which we have outlined above, together with the correct calculations of the data of Haldane, show that deep breathing which is controlled by needs of the body does not alter the dead space or "virtual dead space" of Henderson. The results which we have obtained are what we should expect from data given by E. P. Carter (8) in a paper on the accuracy of the Douglas-Haldane method for calculating the dead space in breathing, in which he called attention to the inaccuracy of the method for obtaining alveolar air when deep expirations were made.

Another interesting point which is brought out by the above experiments is the fact that the Haldane-Priestley method gives oxygen pressures in the alveolar air which are relatively lower and subject to greater error than the CO_2 pressure as determined by the same method. This is demonstrated by a comparison of the figures which Haldane gives for CO_2 and O_2 values in the alveolar air and those obtained by calculation of the same data by the binomial formula. It is also suggested by the fact that the respiratory quotient is invariably lower in the alveolar samples obtained by the Haldane-Priestley method than in the expired air. This observation is of especial interest with regard to the views of Haldane and his coworkers on the oxygen secretion by the lungs at low barometric pressures (9). This subject is being investigated at present in our laboratory.

CONCLUSIONS

1. When the respirations are controlled by the respiratory center and not of extreme depth:

a. The dead space is a relatively fixed anatomical and physiological entity.

b. The dead space for CO_2 and that for O_2 are essentially the same under all conditions.

2. The variations which Haldane, Henderson and their associates find in the capacity of the dead space on deep breathing, and the lack of agreement between the CO_2 and O_2 dead spaces, are attributable to faulty methods and artificial modes of breathing.

3. The Haldane-Priestley method for the determination of the dead space for O_2 or for CO_2 is inaccurate.

4. As great variations in the dead spaces as those found by Haldane and Henderson can be obtained by other methods of altering the respiration than by increasing the depth of breathing.

5. The determination of the percentage of oxygen in the alveolar air by the Haldane-Priestley method is subject to greater error than that for CO_2, and any figures obtained by it for oxygen tension in the alveolar air during exercise or at reduced barometric pressures are untrustworthy.

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THE RESPONSES OF THE CATFISH, AMIURUS NEBULOSUS, TO METALLIC AND NON-METALLIC RODS

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INTRODUCTION

In the course of some experiments on the responses of the common catfish, Amiurus nebulosus (Le Sueur), to mechanical disturbances, the fishes were tested from time to time in a small aquarium of slate and glass by bringing a rod close to them, whereby a slight disturbance was produced in the adjacent water. It was soon found that certain rods were more effective in stimulating the fishes than others. Thus when a piece of heavy galvanized iron wire was brought near a fish, it very usually turned its dorsal fin toward the wire or even swam away as though unduly stimulated, reactions which were not noticed when a glass rod was used. These responses occurred in fishes that were blindfolded and that could not, therefore, rely upon sight to distinguish between the two bodies. As it was a matter of importance to know whether such responses were due to a mechanical disturbance that emanated from the iron wire and not from the glass rod, we undertook to investigate them.

Amiurus is a gregarious fish. A single individual usually swims incessantly and restlessly in an aquarium exhibiting few of the responses easily elicited from any member of a group of such fishes. It was therefore necessary to work with several of these fishes at a time. We found it entirely feasible to use them in pairs, for in pairs they proved to be as quiescent as in larger assemblies and to be individually as responsive as could be wished. To eliminate sight these pairs of fishes were always blindfolded by having their eyes covered with blinders made of thin kid such as is used for ladies' gloves. The blinders,

which had the form of a pair of goggles, were fitted to the surface of the fish's head about the eyes and held in position by being stitched at two places to the animal's skin. Catfishes thus blindfolded soon swam about the aquarium as usual, except that they occasionally collided with its walls; they fed and acted in other respects so normally that there was no reason to suppose that the blinders disturbed them more than to eliminate sight. It was on fishes of this kind that our tests with the rods were carried out.

RESPONSES TO RODS

As already indicated, our first trials were made with a glass rod and a heavy iron wire. When a clean glass rod some 5 or 6 mm. in diameter was carefully introduced into an aquarium and brought near a blindfolded catfish, no response whatever was given to its presence until it was actually in contact with the fish's skin, whereupon the animal was likely to make a lively retreat. If, however, a piece of heavy galvanized wire with a diameter of some 3 mm. was used in place of the glass rod, the fish usually responded by swimming away even when the wire was still some centimeters distant from the creature.

Having ascertained this difference in reaction to the glass rod and the iron wire, we next proceeded to try rods of different materials. Vigorous avoidance responses were given by the fishes to a stout iron rod, a brass rod, a heavy copper wire, a piece of thick fuse-metal, a narrow sheet of block tin and a short amalgamated zinc rod such as is used in a galvanic cell. A slight response was given to a cedar stick 6 mm. in diameter. No response was elicited by glass rods of various diameters from 3 mm. to 7 mm., a celluloid rod, a pine stick and a glass rod covered with a rubber tube. These initial trials showed at once that all the metals tested were stimulating and that the non-metals, except the cedar stick, were without effect provided they did not produce obvious mechanical disturbances in the water.

The fact that the reactions were so generally associated with metals raised the suspicion that these metals might conduct electricity from some outside source to the fish. To eliminate any possible effect of this kind emanating, for instance, from the body of the experimenter, the handle-ends of the various metallic bodies used in stimulation were wrapped with non-conducting materials; but when these metals thus insulated were used again, they were found to be as stimulating to the fishes as before. We then coated the metals with melted paraffin and

thus protected their surfaces against the possible action of the water. On trying them under these conditions it was found that none of them stimulated the fishes. Their stimulating properties, however, immediately returned on scraping off some of the paraffin so that the metal surface was again in contact with the water. From these observations it seemed quite clear that the stimulus from the metal was not due to any of its purely physical properties, such as its elasticity, whereby the water might be set in vibratory motion, but was some disturbance dependent upon direct contact between the surface of the metal and the water.

We next tested a series of metals in a more definite and systematic way. The following were used: aluminum, cadmium, copper, gold, lead, nickel, silver, steel, tin and zinc. From each metal a cylindrical rod was made with a diameter of 3 mm. and a length of about 10 cm.[1] Each rod was fastened by means of sealing wax into the end of a long glass tube. The rod and lower portion of the tube were coated with melted paraffin. To such a coated rod and tube the fishes gave no more response than they did to a plain glass rod. The paraffin was now scraped off all sides of the rod from its free end upward for a distance of 6 cm. As the rods had an approximately uniform diameter of 3 mm., the total area of metal thus exposed was in each instance about 5.6 sq. cm. To all ten rods thus standardized the fishes responded by avoidance reactions. These rods include such a range of metals that it seems fair to conclude that the avoidance reaction will be found to occur in the presence of any metal sufficiently exposed.

The difference between the several metals tested was not very great, but attempts were made to arrange them in a series in the order of their stimulating power. Several such series were worked out, two quite completely. In the first of these the metals fell in the order: steel > Cu = Sn > Zn > Au = Ag = Al > Cd = Pb > Ni. In the second the order proved to be steel > Cd > Au > Zn > Ag > Pb = Cu = Al > Sn > Ni. A comparison of these and of other tests showed that the significant features of the two series given were really only two; first, that steel was always the most stimulating and, secondly, that nickel was always the least so. To the other metals the fishes responded with such varying reactions that no definite posi-

[1] We are under obligation to Prof. G. W. Pierce for many suggestions and much assistance, particularly for help in the preparation of the metallic rods and the use of a portable d'Arsonval galvanometer.

tions could be assigned them excepting that they were generally not so stimulating as steel and more so than nickel.

We next attempted to ascertain the effect of exposing more or less of the metal to the water. To a rod of steel free to the water for 6 cm. of its length (area about 5.6 sq. cm.), all the fishes in a considerable group in the aquarium responded by swimming away. When only 3 cm. of length was exposed thus reducing the area by half, the fishes commonly turned toward the rod and often nibbled it or bit at it. The nibbling response was most striking and interesting for it reproduced in all respects the reaction of a fish taking a natural bait. It was sometimes hard to believe that the rod was not contaminated with organic matter. When 1 cm. of rod was exposed the reactions were very few, but if they occurred at all, the fishes commonly turned toward the rod and sometimes nibbled it. When the rod was completely covered with paraffin, no more response was made to it than to a rod of glass. Similar reactions were observed when copper was used in place of steel. We therefore concluded that considerable areas of metal, such as 5.6 sq. cm., induced negative responses; smaller areas, 2.8 sq. cm. or even 0.9 sq. cm., positive movements.

We next endeavored to find out what it was about the metals that stimulated the fishes. It is conceivable that the surface of the rod gives off material particles that travel through the water to the fish, which is thereby stimulated. Such particles must travel at relatively high speed and must be vigorously stimulating. Hydrogen ions come nearest to fulfilling these requirements. To get some clue as to whether or not small amounts of hydrogen ions were stimulating the fish, we immersed the fish in a weak acid solution to determine if the hydrogen ions present in such a solution would mask the reaction. This operation was carried out in the following way. Two fishes were put in a large, clean glass jar containing 15,000 cc. of tap water. On being tested they were found to be very responsive to an iron rod. Hydrochloric acid was now added to the tap water in which the fishes were, till a concentration of $\frac{N}{10000}$ was obtained. After having been ten minutes in this solution, which was apparently not stimulating to the fishes, they were tested again with the iron rod and found to react at once by avoidance movements. The solution in the jar was now strengthened to $\frac{N}{2000}$ hydrochloric acid and, after a period of ten minutes' rest, the fishes were again tested and responded again to the iron rod. It is thus certain that a low concentration of hydrogen ions does not mask the reactions of these fishes to metals and hence such particles are not to be regarded as the effective elements in stimulation.

Another way of ascertaining whether or not material particles are given out by the rods is to coat the rods with agar. A thin layer of this substance on the outside of the rod will restrain for a considerable period of time any material particles that might emanate from it. We therefore used agar as a coating for the metal rods. When a steel rod to which the fish had been responsive is coated with agar to the depth of about 1.5 mm., it still remains stimulating to the fish, though this property at once disappears on coating the rod with paraffin. The same was true of several other metals that were tried. It therefore seems probable that the stimulus from the rod was not due to material particles; but to guard against any possible mishap, such as perforation of the coating of agar, this experiment was repeated in another way.

A parchment-paper diffusion tube 10 cm. long and 2 cm. in diameter was soaked in water till it was soft, after which it was filled with agar. On introducing this tube among the fishes, no response was observed. The standard steel rod was now inserted into the axis of the agar-filled tube and additional agar was added at the top of the tube so as to fill it completely. Thus the rod was unquestionably coated on all sides with a considerable thickness of agar and in addition was in a tube of parchment-paper. Yet the fishes responded at once to the tube and rod when it was lowered into the aquarium. This experiment was repeated several times and always with the same results. It therefore seems clear that the stimulus that emanates from the metals and affects the fishes cannot be in the nature of material particles, but must be some form of disturbance that can be propagated almost instantly from the given metal through the agar and water to the fish.

This view of the nature of the stimulus is in full accord with the fact that a quiet, blindfolded fish will respond to a large metal rod the moment it is introduced into the aquarium and before it has come within 10 cm. of the fish, a state of affairs quite inconsistent with the assumption that material particles passing from rod to fish are the means of stimulation.

Having determined that metallic bodies probably stimulate catfishes through disturbances generated by their contact with water, we next turned to the only non-metallic body that had been found to be stimulating, namely the cedar rod. A cylindrical rod of red-cedar wood 6 mm. in diameter when brought near the head of a catfish is usually responded to by a slight avoidance reaction. If the rod is coated with paraffin the reaction fails to appear, as in the case of metals so coated. If it is coated with agar, the reaction also fails to appear. This condi-

tion led us to conclude that the stimulus from the cedar rod is essentially different from that from a metal and must consist of material particles, for it is checked by a coating of agar. The cedar stimulus is also different from the metallic stimulus in its regions of effectiveness. A small metal rod will stimulate a fish when held near any part of the animal's body, but the cedar rod is effective only near the head end and especially immediately in front of the fish. Since the wet cedar has a very strong odor, we suspected that its stimulating qualities were due to odorous particles which reach the nostrils of the fish. This suspicion was confirmed by finding that fishes whose nostrils had been temporarily stitched up no longer responded to the cedar rod though they were still normally affected by a metallic rod. We, therefore, believe that the stimulus from the cedar rod is wholly unlike that from the metals and is dependent upon material particles. No other non-metallic bodies tested exhibited the same qualities as the cedar wood did, but many doubtless could be easily found such as meat, earthworms and other bodies that serve as odorous baits.

In conclusion, then, it can be stated that the rods worked with were capable of producing at least three kinds of stimuli: mechanical stimuli, which were characteristic of all rods tested; olfactory stimuli from such as the cedar rod; and stimuli non-material in character for all metallic rods.

RESPONSES TO ELECTRIC CURRENTS

Non-metallic bodies that affect catfishes at a distance probably always do so in consequence of the material particles that they give out. What is the precise character of the influences that emanate from metallic bodies? The fact that these influences stimulate the fishes at once and at a considerable distance and that they are intercepted by paraffin suggested that they are electric. That they are not associated with the body of the experimenter has already been made clear. Nor do we believe that they are in any way dependent upon the interaction of the rod with the small amount of metal used in the construction of the aquarium the walls of which, as already mentioned, were of glass and slate cemented together. Moreover the same reactions were obtained from fishes in a large glass jar whose walls were absolutely without metal fittings. That these reactions are not due to an electric charge on the metallic rod itself can also be easily shown by grounding the rod. If a particular piece of metal is grounded by having a wire run from it to a water pipe reaching into the soil, it is still as stimulating to a fish

as it was in its ungrounded state, showing that the stimulus is in no sense dependent upon an electric charge. Frictional electricity generated by rubbing a glass rod with silk or a piece of sealing wax with flannel was also found to be without observable effect upon fishes. This negative evidence led us to consider the possibility of metallic stimulation as the result of currents produced locally on the metallic rods themselves by the action of tap water. With this general view in mind, we attempted to imitate the effects of the metals by direct weak currents introduced into the aquarium by appropriate electrodes.

A Columbia dry cell of 1.57 volts was set up so that the wires from its poles could be used as electrodes in the aquarium. Between the aquarium and the cell both wires were interrupted by a double knife-blade switch. The ends of the wires to be used as electrodes were mounted in a long glass tube as a waterproof handle. The tips of the wires, which projected from the tube some distance, were kept about 2 cm. apart, and the insulating covering was removed from them for a distance of 1 mm. thus exposing in all 2 mm. of fine copper wire.

When these electrodes, without current, were introduced among a group of fishes in the aquarium, no disturbance whatever was noticed, showing that the amount of copper exposed had no appreciable influence on the fishes. If while the electrodes were near the fishes, the current was made at the switch the fishes scattered in all directions as though excessively stimulated. It was evident from these preliminary trials that the current coming directly from a Columbia cell was much too strong for critical observations on catfishes.

To reduce this current a resistance was inserted on the line of each wire between its electrode and the switch. This was made by cutting the wire and inserting between the two cut ends a long glass tube filled with distilled water. Into the lower end of the tube was sealed one end of the wire and into the upper end the other end of the wire was slipped so that it could move freely up and down and recede from the attached wire or approach it, thus varying the resistance.

When a resistance of 65 cm. of distilled water in each tube or a total of 130 cm. in all was used, no reaction was noticed as the electrodes were brought near to the fishes. When the resistance was reduced to 1 cm. of water in each tube or a total of 2 cm. for both, the fishes always turned vigorously away from the current. Observations were therefore carried on between these two extremes and the records of the reactions of the fishes to the varying amounts of current expressed in microamperes are given in table 1.

For each strength of current the fishes were tested with the electrodes as they had previously been stimulated with the metal rods. Immediately after the reactions of the fish to a given strength of current had been determined, the electrodes were attached to the poles of a galvanometer and the five readings given in the table were taken; from these the amount of current in microamperes was calculated. The galvanometer used was a Leeds and Northrup portable d'Arsonval, 1 mm. of the scale of which was equal to 0.182×10^{-6} ampere or 0.182 microampere.

TABLE 1

Reactions of Amiurus to direct electric currents varying from 2.45 to 0.67 microamperes

RESISTANCE IN CENTIMETERS OF DISTILLED WATER	REACTIONS OF FISHES	GALVANOMETER READINGS IN SCALE MILLIMETERS					AVERAGES	CURRENT IN MICROAMPERES
cm.								
2	Strongly negative	13.5	13.5	13.5	13.3	13.6	13.48	2.45
10	Negative	10.0	10.1	10.1	10.1	10.0	10.06	1.83
10	Negative	9.2	9.4	9.4	9.3	9.3	9.32	1.70
10	Negative usually, positive rarely	8.2	8.1	8.0	8.1	8.0	8.08	1.47
27	Negative sometimes, positive sometimes	6.2	6.3	6.2	6.1	6.1	6.18	1.12
36	Positive, often nibbling	5.5	5.5	5.4	5.4	5.4	5.44	0.99
72	Slightly positive, nibbling	4.5	4.7	4.6	4.6	4.6	4.60	0.84
72	Slightly positive	3.9	4.1	3.9	4.0	4.0	4.38	0.80
72	Slightly positive, nibbling	3.7	3.6	3.7	3.8	3.7	3.70	0.67

With a total of 2 cm. of distilled water as resistance the current was 2.45 microamperes and from this the fish turned away with great vigor. At a total of 10 cm. resistance the current varied in different trials from 1.83 to 1.47 microamperes, depending upon the varying condition of the distilled water used as a resistance, and to this the fishes were generally negative. When the current was reduced to 1.12 microamperes, the fishes turned sometimes sharply toward the electrodes and at other times away from them. This condition is not indicative of indifference to the current on the part of the fish, for when no current is flowing the fishes usually swim past the electrodes without turning either one way or the other, a true indication of indifference. With a current of 1.12 microamperes sharp turns were made either toward the electrodes or

away from them, thus giving unmistakable evidence of stimulation. At 36 cm. resistance, with a current measuring 0.99 microamperes, the fishes turned sharply toward the electrodes and often bit at them and nibbled them as they did the rods of metal when 3 cm. of length was exposed. It was extremely interesting to observe these positive responses. Often the fishes nibbled at the region of the current between the electrodes exactly as though they were taking a bait of worm, meat or other such body, though there was no solid material there. At a resistance of 72 cm. of distilled water the current varied from 0.84 to 0.67 microamperes and to this the fishes were generally positive and exhibited many nibbling responses. To less current than 0.67 microampere little if any reaction was given. Thus a direct current calls forth practically the same range of responses that the rods did: negative responses to a strong current as to a rod much of whose surface is exposed and positive responses to a weak current as to a rod little of whose surface is exposed. It therefore seems probable that the rods stimulate through the electric currents they themselves produce.

That the current from such a source as a Columbia cell does not stimulate through particles that it causes to emanate from the electrodes can be shown to be highly probable by employing the tap water of the aquarium as an electrode, so to speak. If instead of using directly metal electrodes of fine copper wire for stimulation, the electrodes are inserted each into the outer end of a glass siphon tube through which aquarium water is flowing freely from the aquarium and the two siphon tubes are bound together with their central ends 2 cm. apart, there results a device which will allow a current of electricity to flow from the inner end of one tube through the aquarium to that of the other tube without contamination from the metal itself, for the metal will be continuously washed by the outflowing water. When the ends of such a pair of tubes were brought near a fish, no response was made, but the moment the current was switched on the fish turned sharply away. As the ends of the wires in the siphon tubes were each fully 40 cm. from the inner ends of the glass tube and as the outward-going current of water was running at the rate of 30 cc. per minute, it is inconceivable that material particles could be liberated from the metal and make their way against the water current into the aquarium.

Nevertheless we sought other methods of stimulating the fishes electrically with the avoidance of material particles and finally found one in which all possibility of material influence was eliminated, though this was not with a direct current. Into a large glass tube 40 cm. long,

9 cm. in diameter, and closed at one end, was inserted an electromagnetic coil through which an ordinary alternating lighting current was sent. By this arrangement an induced current could be developed in the surrounding aquarium water notwithstanding the fact that the metallic generating apparatus was entirely cut off from the water by glass.

When the large glass tube containing the electromagnet was put into the aquarium, it could be moved about over the blindfolded fishes, as any other glass object could, without disturbing them. If when it was in position over a group of four or five quiet fishes the current was made, the fishes immediately roused, moved about and quickly swam away. This form of experiment was repeatedly tried and with a uniform outcome. There can be no doubt that an electric current of itself and unassociated with special material particles will stimulate a fish just as a metal rod surrounded by agar will. Thus the rod stimulus and the current stimulus agree in that they occur independently of special material particles and each calls forth from the fishes in a quantitatively graded way the same series of rather complex and diversified reactions.

CAN ELECTRIC CURRENTS BE IDENTIFIED ON THE METALLIC RODS?

When a piece of metal is immersed in tap water, electric currents are generated in consequence of the action of the water and its contents on the metal and its impurities. These currents flow in a given direction through the water and return in the reverse direction through the metal. It is our belief that these currents as they pass through the water in the neighborhood of the rod stimulate the fish. We, therefore, attempted to identify and measure them.

One way of accomplishing this end was to cut the stimulating rod of metal in two and conduct the current from one half to the other through a galvanometer. Such a procedure made of the rod a pair of polarizable electrodes whose combined currents were the currents we sought to measure. The details of the operation were of the following kind. The actual metal rods with which we stimulated the fishes were each cut transversely in halves. To one end of each half-rod a copper wire was attached and that end was coated with melted paraffin so that 3 cm. of length measured from the opposite end was left exposed. The two half-rods were then put in position on a small paraffin stage in a shallow glass vessel filled with aquarium water. The half-rods were

placed end to end and were separated by a small plate of mica which served as a non-conductor. The wires coming from the half-rods were led through mercury switches to a galvanometer. In this way it was possible to gain some idea of the nature of the currents on the metal rods. These currents, as might have been expected, were quite irregular. Most rods when first introduced into the tap water produced currents of relatively much energy, which fell rapidly till a fairly constant current of slight energy was established. Thus in one test when the half-rods of lead were first immersed, their current measured 5.7 microamperes. In the course of 27 minutes this sank by regular steps as shown by readings on the galvanometer taken once a minute, to 0.9 microampere. Here it remained with fair constancy never rising above 1.1 microamperes nor falling below 0.6 microampere for a quarter of an hour when the observations were discontinued.

An attempt was then made to ascertain whether the currents varied with the area of the rod exposed. A pair of zinc half-rods were seasoned in tap water for half an hour and their combined currents measured when 6 cm. of length (5.6 sq. cm.), were exposed. The half-rods were then dipped in melted paraffin till only a total of 3 cm. of length was left uncovered (2.8 sq. cm.). In the former case the current measured 0.85 microampere; in the latter 0.44 or a little over half of the first amount. A pair of steel half-rods similarly treated gave 1.32 microamperes for the total area and 0.65 microampere for the half area. These observed results are so close to what might have been expected that we conclude that in the case of any metal the currents are proportional to the metallic surface exposed when that surface is of appreciable extent.

The observations described in the preceding paragraphs support very fully the contention that the currents produced by the rods are their means of stimulation for, as we saw in the beginning, a rod when exposed over 6 cm. of its length is much more stimulating to a fish than when it is exposed over only 3 cm. And we now know that there is about twice as much current produced in the former condition as in the latter.

The powers of the different rods to generate currents were also tested. In order to compare the several kinds of rods that we had used in stimulation, the half members of each pair were thoroughly cleaned, then seasoned by being immersed in tap water for half an hour and finally measured for their currents by the galvanometer. Ten readings were taken for each metal and the condensed records are given in table 2, in which two such sets of measurements are included.

TABLE 2

Two sets of measurements in microamperes of electric currents produced by ten pairs of metal half-rods in aquarium water

		KINDS OF METAL RODS								
		Steel	Ag	Sn	Al	Au	Cu	Zn	Pb	Cd	Ni
Currents in microamperes...	First set.....	2.00	0.43	0.37	0.35	0.24	0.23	0.21	0.13	0.10	0.02
	Second set....	1.57	0.12	0.12	0.43	0.20	0.30	0.37	0.41	0.41	0.02

An inspection of the table shows great diversity in the relative positions of the metals in the two sets of records. But in both sets steel is highest and nickel is lowest and with margins that establish clearly their extreme positions. The other eight metals lie much more closely together and give no evidence of constancy of position. Silver, which is second in the first set, is eighth in the second, and cadmium, which is ninth in the first set, is fourth in the second. Copper is sixth in both sets, but this must be a mere coincidence.

The significant feature of these determinations is the high place of steel and the low place of nickel. In other tests which included only three metals this mutual relation was retained as is shown in table 3.

It will be recalled that the series of metals established on the basis of the stimulating power of the standard rods exhibit much the same peculiarities that are shown in tables 2 and 3. In these series it will be remembered that steel was always most stimulating to the fish, nickel least, and the other metals including copper were irregularly intermediate. This comparison therefore gives support again to the view that the metals in contact with tap water stimulate in consequence of the currents they produce.

If a metal rod in tap water produces electric currents that stimulate a fish, such currents ought to be identifiable in the water about the rod

TABLE 3

Six sets of measurements in microamperes of electric currents produced by three pairs of metal half-rods in aquarium water

	NUMBER OF SET					
	1	2	3	4	5	6
Steel.....	2.00	1.14	0.46	1.23	1.43	1.57
Copper.....	0.23	0.25	0.39	0.23	0.49	0.30
Nickel.....	0.02	0.05	0.06	0.02	0.02	0.02

at a position corresponding to that in which the fish is stimulated. Apparatus was therefore set up in such a way as to duplicate exactly the conditions under which the fishes were stimulated. Two fine copper wires from the two poles of the d'Arsonval galvanometer were led directly into a glass basin containing aquarium water. They were fixed about 3 cm. apart and represented the fish. As a stimulating metal we chose a cylindrical rod of zinc such as we had used in our preliminary experiments. This was of the kind commonly employed in galvanic cells; it had a diameter of 9 mm. and was one of the most, if not the most stimulating piece of metal that we had used. When the two copper wires were in the aquarium water, the galvanometer showed a slight current due to their polarization. This current was small in amount and fairly constant. While it was being watched on the scale of the galvanometer the zinc rod was introduced into the tap water and made to approach the electrodes as it had been used with the fishes. Immediately the current changed, and it fell back to the original reading only on the removal of the zinc from the water. The amount of current identified in this way on several determinations was never over 0.015 microampere, but it was very clearly and definitely observable even when the zinc rod was 1 cm. from the copper terminals. On dipping the zinc in paraffin and trying the same test with the coated rod, no change in the polarization current was to be observed. Hence we believe that we have identified the stimulating current.

We next tried to detect in a similar way the currents from the standard rods. When a rod of steel exposed for 6 cm. of its length (area 5.6 sq. cm.) was made to approach the electrodes in tap water, perfectly clear evidence of a very slight current, scarcely measurable, could be made out in the galvanometer. When a rod of nickel was tried in the same way, no sign of a current could be seen. Probably in this case the current was too slight to affect the galvanometer, but with the more active pieces of metal, the zinc and the steel, the identification of currents in the adjacent water was beyond doubt. We therefore believe that we tapped the current by which the fishes are stimulated.

Although the stimulating current as measured in the half-rods is very slight, at most only a few microamperes, that which affects the fish must be in reality very much less, for stimulation occurs well out in the periphery of the currents. It is probable that we are dealing here with one of those attenuated forms of stimuli comparable to that which Nägeli discovered in water that had been temporarily in contact with certain metals and whose action he designated as oligodynamic.

THEORETICAL

How metals in tap water produce electric currents is perhaps a question in physical chemistry rather than in biology. It is probable, however, that the currents result from the action of the water and its dissolved materials upon the metallic surface with its included impurities. At least when the metals are tested in distilled water in place of tap water their currents are always greatly reduced. Thus the pair of zinc standard half-rods that showed a current of 0.33 microampere in tap water were found on being transferred to distilled water to produce a current of only 0.01 microampere. We suspected, therefore, that with a very pure metal in a very pure water such currents might be made to disappear almost entirely. Through the kindness of Prof. G. P. Baxter we obtained two half-rods of zinc known to contain not over one part of impurities in ten thousand. On trying these in tap water we found a current of 0.22 microampere which fell to 0.004 microampere in ordinary distilled water. This amount of current was easily recognizable and we were forced to conclude that if the impurities are essential to the current, only a mere trace of them in a metal is sufficient to give rise to these currents. It is interesting to observe that the fishes were about as much stimulated by this sample of very pure zinc as they were by the standard rod of commercial zinc with which we had tested them originally.

We have not attempted to ascertain with certainty what receptors on the fishes are stimulated by the electric currents from the metals. That they are in the skin of these animals seems highly probable, for if the skin of a catfish is treated for five minutes with a 20 per cent solution of magnesium sulphate, all traces of electrical as well as other receptivity disappear.

The fact that the responses to metals are most pronounced when these bodies are made to approach the head of the fish, though they may be elicited when the metal is held near the flank, suggests that the receptive organs concerned are the taste buds, for these are abundant about the head and less numerous along the flanks. Aside from these general indications, however, we have no grounds for any determination as to the exact sense organ concerned.

If, as we suspect, the organs of taste are the receptors stimulated by the currents, the nibbling responses of the fishes to rods of small exposure are readily explainable, for it is chiefly through the gustatory organs that feeding activities are excited. This assumption is completely in

line with what has long been known of human taste organs for these are easily stimulated by direct electric currents of very low energy value.

That a catfish may be induced to nibble a small piece of metal in water in consequence of the electric currents it produces, is at first sight of no great significance, but when it is remembered that these fish are commonly caught with metallic hooks, it is not a far reach of the imagination to think of hooks constructed of such surface exposures as to serve both as bait and holdfast. If, however, it should prove impractical to accomplish both purposes, it is conceivable that the hook might be attached to an insulated wire in place of the ordinary fish line and thus provided with an appropriately slight current from an electric cell in the hands of the fisherman, the return current coming back to an electrode in the water near the operator. Thus an unbated hook might be made so attractive to a fish as to serve as a means of catching it. But whether fishing with electricity could be applied to fishes other than the catfish or is in fact anything more than a pure fancy, remains to be discovered.

SUMMARY

1. To rods of any kind blindfolded Amiurus responded regularly when the rod touched the skin of the fish.

2. To a rod of cedar wood, which contains odorous materials, blindfolded Amiurus responded at some distance through the olfactory organ to the material particles that emanated from the rod.

3. To metallic rods (Al, Cd, Cu, Au, Pb, Ni, Ag, Fe, Sn, Zn) blindfolded Amiurus responded even when a given rod was some centimeters from the fish. When much of the rod was exposed to the water, the fish swam away from it; when little was exposed, the fish swam toward it and often nibbled it.

4. These responses are not due to vibrations set up in the water by the metallic rod, nor to particles emanating from it, nor to electrical disturbances coming from the experimenter, nor to an electric charge on the rod itself, nor to a reaction between the rod and other pieces of metal in the aquarium.

5. These responses can also be called forth by a direct electric current; to such a current of 1 microampere or more Amiurus usually responded by swimming away; to one of a little less than a microampere down to about 0.7 microampere, it commonly responded by approaching the current and nibbling.

6. These currents do not stimulate by means of material particles given out from the region through which they pass.

7. The metallic rods in contact with water generate on their surfaces electric currents of the same order of magnitude as those used to imitate the action of these rods. It is the currents thus produced on the surfaces of the rods that make the rods stimulating to the fishes.

8. The organs of the fishes stimulated by these currents are very probably the gustatory organs.

ON THE HEAT LIBERATED BY THE BEATING HEART

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INTRODUCTION

The determination of the heat evolved by the beating heart was undertaken with a realization that the results would be of importance first as an addition to our special knowledge of the heart's physiology and second as a help in the solution of the general problem of the nature of muscle contraction.

The effort to measure the heart's heat is not new, for Herlitzka (1) has already published a paper on a similar investigation. This author made his observations upon the isolated rabbit's heart fed with various perfusing fluids. The thermoelectric method was used, a modification of Bürker's "umfassende" thermopile and the thread galvanometer with a resistance of 5300 ohms being employed for the purpose. Herlitzka himself seems fully to appreciate the difficulties of and the objections to the method and the instruments used. His records show a very small thermoelectric response. The rate of the warm-blooded heart is so rapid that one doubts whether there could be time enough for the conduction of heat to the thermoelectric junctions, time enough to be able to tell anything about the absolute quantity per beat or about the point of time in the cycle wherein the heat first appears. In using the "umfassende" thermopile one is never sure that it does not slip over the surfaces of the tissue while the muscle shortens, unless it is in an isometric contraction or where practically no movement of the tissue takes place. Such a condition would be hard to obtain in a beating heart. The very fact that the heart chambers were filling and emptying themselves of the perfusing fluid would seem to make it certain that the walls of the heart were now tightly pressed against the thermopile junctions, now pulling away leaving them free.

Bernstein (2) has cast doubt upon Herlitzka's results saying that the thread galvanometer with its great resistance could not be sensitive

enough to respond to the small thermoelectric current that might arise during the heart's beat. He is inclined to regard the electrical response obtained by Herlitzka as the action current of the heart itself which, through some defects in the insulation of the thermopile, leaked into the galvanometer circuit. But this possibility had been considered in the original paper and as is pointed out in the reply to the criticism (1), (1913) the form of the curve in the photographic record, were it an electrocardiogram, would be quite different from the one obtained.

From the data published one sees that the difference in time between the beginnings of the mechanical heat curves varies from 0 to 0.10 second and that in some cases the heat curve precedes that of the mechanical curve as much as 0.02 and 0.05 second. The heat is all practically produced during the systole of the heart-beat, so far as one can tell from the records.

METHOD AND MATERIAL

In view of the many doubts that arise in regard to the results obtained with the isolated rabbit's heart, the "umfassende" thermopile and the thread galvanometer, it was thought worth while to investigate the matter using a different heart and different instruments.

In order to be able to make an observation of the heat evolved by a single heart-beat it is obviously necessary to have an interval of time between the beats sufficiently long to allow the recording apparatus to register the accompanying rise of temperature. For this purpose instead of the heart of a warm-blooded animal that of a cold-blooded animal may be used. In the present investigation the heart of the terrapin was used. To slow its rate the temperature of the moist-chamber was kept at 10° or 12° C. Partial block also suggested itself as a means of further lowering the rate of the ventricle. In two of the experiments that form the basis of this paper the heart, by the preliminary manipulation, sustained apparently just enough injury to the conducting substance to establish a permanent partial block.

For heart-chamber a "thermos" food jar, a form of Dewar vessel obtained in the shops, was used. This was fitted with a thick hard-rubber cover through which all the necessary connections with the outside could be made. By placing ice in the bottom of the jar its temperature could be kept at the desired degree for a period of time quite long enough for an experiment.

With the heart rate diminished to a rate of five beats per minute one can choose another form of galvanometer instead of the less sensitive

thread galvanometer. The Paschen galvanometer with its low resistance, its comparatively short period of oscillation, its almost complete aperiodicity, and above all its great sensitivity, was the instrument chosen. The disadvantages of the Paschen are the liability to uncontrollable magnetic disturbances and the impracticability of photographic registration of its deflections. In the present case street cars in the vicinity (100 meters away) were the chief cause of magnetic disturbances. By doing the experiments between one and four o'clock in the morning when the car service was reduced to one car every thirty minutes, satisfactory observations could be made. In lieu of the photographic registration a method of signaling the deflections of the galvanometer mirror upon a revolving drum had been developed that also proved quite satisfactory.

Thermopiles of two forms were built of constantan and iron wires about 0.12 mm. diameter and 11.5 mm. lengths with sixty junctions to the pile. One of the instruments was made after the "umfassende" type of Bürker which we may call the clasp-thermopile, the other was made after the type of Bürker's "Gittersäule," which we may call the insert-thermopile. The chief difference between these two instruments is that in using the one the instrument is made to clasp the tissue, in using the other the tissue is made to clasp the instrument. In adjusting the clasp-thermopile to the beating heart one very soon becomes convinced that there is no position in which one can fasten the instrument about the heart and feel certain that it will remain so without slipping. If the heart wall pulls away from some of the junctions regularly with every beat the effect will be as if a cooling takes place, and if this pulling away process is followed by one making a close contact with the junctions the effect will be as if a warming takes place. One can well imagine that these two acts may occur with each contraction and relaxation of the ventricle and the picture of the heat production that one may then observe would be just what one expected, a production of heat during systole and an absorption of heat during diastole. But clearly such heat changes would be artifacts and have nothing to do with the real heat changes going on within the muscle fibers themselves, the heat changes that we are trying to discover and observe.

In order to avoid this possible source of error it was decided to split the ventricle from the tip of the frenum (that is, lengthwise through its tendon) through apex to base and place an insert-thermopile between the two halves. The halves of the ventricle are thus used like a pair

of frog's gastrocnemius muscles astride a "Gittersaüle" thermopile. Threads tied to the two strands of the frenum enable one to fasten the thermopile securely in place and at the same time to make connection with a writing lever.

The resistance of the insert-thermopile as used was about 14.5 ohms. Its orignal resistance was 11.0 ohms, but repeated rewrapping and polishing the wires with emery paper reduced their diameters and thus increased the resistance. The resistance of the clasp-thermopile was nearly 11.32 ohms. The frame of the instrument is made up of the two halves of a circular flat disk, one millimeter in thickness, the two halves make up a flat ring, as they are held together at one end by a hinge and at the other by an adjustable clasp or latch. The junctions of the wires are not distributed at equal intervals over the whole of the ring but in two sets of 30 junctions each, each set occupying about 120 degrees of the arc of each half circle. The hinge and latch each cover about 60 degrees of arc. The adjustable feature of the latch enables one to fit the instrument to muscles of various diameters. The weight of the whole instrument is nearly 2.4 grams. When in use it is suspended from the top of the thermal chamber with delicate spiral springs.

In estimating the values of the galvanometer deflections in terms of temperature change it was decided to use the theoretical thermoelectric power of the thermopile as the basis. The direct method of calibration whereby the muscle used in the experiment is finally killed and then heated with a known quantity of electricity has the objection that the electrical current in passing through the dead muscle may activate or accelerate oxidations in the disintegrating tissues that in themselves may give rise to unknown quantities of heat and thus vitiate the value of the galvanometer calibration. Until this possible source of error has been investigated it has been thought best at least in the present study to content one's self with the theoretical thermoelectric power of the thermal junctions.

A convenient formula for the rapid calculation of the value of the galvanometer deflections in terms of degrees temperature is the equation

$$\Delta t = \frac{r_1 + r_2}{n \cdot p \cdot m} \cdot i,$$

where

Δt = the rise or fall in degrees C. represented by one scale division deflection of the galvanometer,

r_1 and r_2 = the resistances of the thermopile and galvanometer respectively when used in series,

n = the number of elements in the thermopile,
m = the total deflection of the galvanometer to the calibrating current, in scale divisions,
i = the strength of the calibrating current in amperes,
p = the thermoelectric power of one thermal element in volts.

In the experiment of June 22, 1917, for example, the sensitivity of the galvanometer was 30 s. d. to a current of $8.3 \times 10.^{-8}$ amperes. The resistances were 14.5 and 12.2 ohms respectively. The thermopile was made up of 30 pairs of junctions each having thermoelectric power of 53×10^{-6} volt. Substituting we have

$$\Delta t = \frac{14.5 + 12.2}{30 \cdot 53 \times 10^{-6} \cdot 30} \times 8.3\ 10^{-8} = 4.6 \times 10^{-3}\ °\text{C}.$$

The observations during the experiments were recorded upon a revolving drum, the time marker being a Jacquet, marking seconds. The mechanical movements of the ventricle were recorded by a Ludwig (chord describing) lever. The deflections of the galvanometer were recorded by means of a pneumatic signal. This signal is preferred to the electromagnetic signal on account of the disturbing influence the latter has upon a galvanometer of the Paschen type. Practice with the signal soon gave one skill enough to follow the comparatively slow events satisfactorily. A preliminary test was made with the signal to record the galvanometer deflection in response to a small current in intervals of 10 mm. of scale. In this the closing of the circuit was signaled by the pneumatic signal. The following table shows the delays for the first three successive intervals:

TABLE 1

NUMBER OF TRIAL	TIME IN SECONDS TO REACH THE		
	FIRST 10 S. D.	SECOND 10 S. D.	THIRD 10 S. D.
1	0.5	0.7	0.8
2	0.5	0.6	0.6
3	0.3	0.5	0.35
4	0.35	0.4	?

The production of heat in the heart muscle, and therefore the thermoelectric current arising from the heart, doubtless has a velocity of production quite different from the velocity of development of the calibrating current used in the above test (2). In the latter the full force of

the current is developed instantaneously and thrown into the galvanometer at once, in the former case the velocity of the current development more probably follows the velocity of a chemical or enzyme action, and therefore increases with the time comparatively slowly.

What the control test brings out is that the pneumatic signal is quite adequate to follow the deflection caused by a small current of the magnitude of that of a muscle contraction.

Indeed it was later found that intervals of 5 mm. of scale deflection could be easily recorded by the signal. The test further indicates that the period of the galvanometer under the chosen degree of damping is not greater than two seconds.

The operator's reaction time is very small compared to the times here dealt with and may safely be neglected.

The preparation of the heart for experiments involved no special treatment excepting in the cases where the insert-thermopile was applied. For this, as already stated, the ventricle was split dorso-ventrally from tip of the frenum to the atrio-ventricular groove. After isolating the heart entirely from the body it was suspended by the great aortae to the fixed point of support of the moist-chamber. The thermopile was then laid in between the two halves of the ventricle and held in position by tying the two strands of the frenum together again. The thread used for this purpose was then led up to the muscle lever which in turn transmitted the movements of the ventricle to the revolving drum.

In all cases the heart was not perfused but allowed to hang freely in moist air. The objection raised by Bernstein (2), that with each beat fluid of a different temperature bathes the tissues under the warm

Fig. 1. This figure is one of the drum records taken during the experiment of June 22, cut into three parts. The parts are placed under each other and are separated by the white horizontal spaces. The beginning of the middle part should therefore be read as a continuation of the upper part and that of the lower as a continuation of the middle section of the record.

As to the tracings in each section, there are two sets each made up of the following:

a. A lower tracing of the Jacquet chronograph marking seconds. *b*. A middle tracing of the pneumatic signal marking 5 mm. deflection of the galvanometer. The high upstrokes mark deflections in the direction of rise of temperature, the lower upstrokes mark deflections in the directions of falling temperature. *c*. An upper tracing of the muscle lever attached to the terrapin's ventricle, showing the usual tracing of a surviving heart. The partial block in the beat is seen by the small atrial beats occurring at regular intervals.

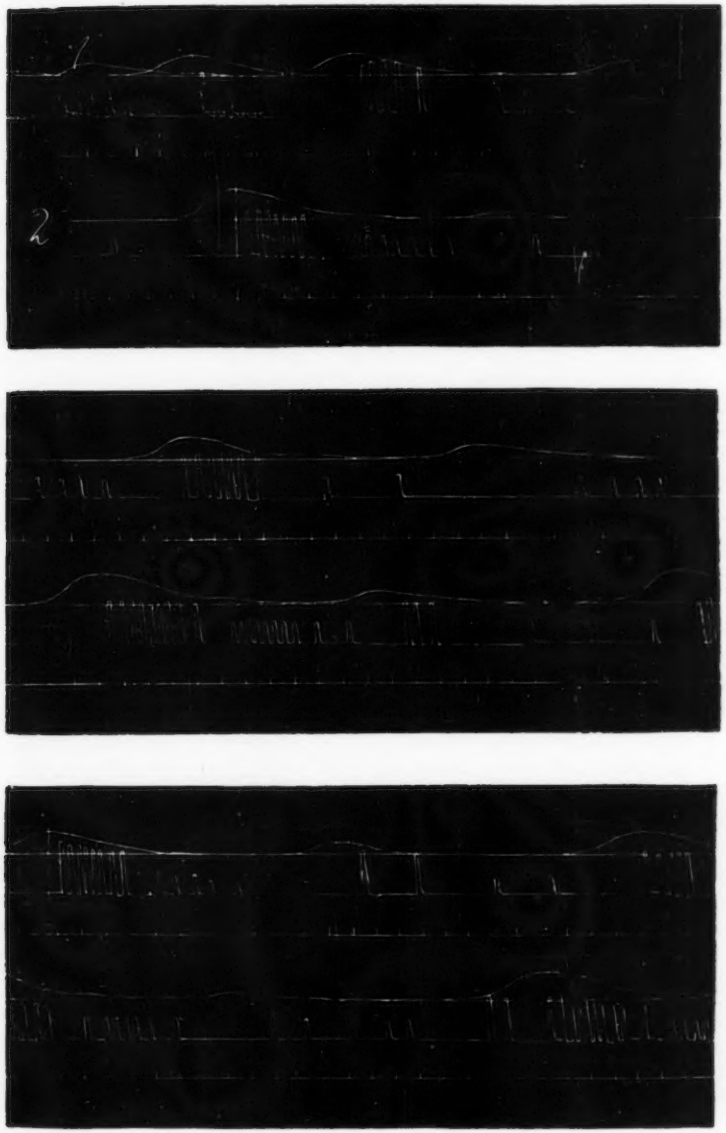


Fig. 1.

junctions and thus may give rise to temperature change, does not apply in these experiments.

The cold junctions are covered with moist strips of filter paper and the moist-chamber is kept cool with ice placed an hour previously in the bottom. It requires from a half to one hour after the heart is thus enclosed in the moist-chamber for the cold and warm junctions to show a temperature equilibrium.

RESULTS OF EXPERIMENTS

Experiment of June 22, 1917. The results of this experiment will be given first because the experiment as a whole proved most successful. As already stated the heart in this case was in a state of partial block, so that the rate was slower even than would be expected if only the decreased temperature were affecting it.

The heart was isolated and mounted in the moist-chamber between 11.30 p.m. and midnight, and observations were begun about 1.00 a.m. These were continued until about 4.00 a.m. The records taken after 3.10, however, showed a weakening of the force of the beat, an increasing irregularity and finally a tendency to fibrillation. The records taken after 3 o'clock, therefore, are not included in the present study.

The other records show throughout a certain grouping of alternate strong and weak beats, characteristic of partial block. The well marked galvanometer deflections occurring regularly with the strong ventricular beats stand in sharp contrast beside the absence of such deflections during the weaker atrial beats (see fig. 1).

Toward the end of the experiment control records were taken. In these the galvanometer was switched off from the thermopile and all the movements of the mirror recorded beneath the heart-beats as in the former records. If the rhythmical deflections of the galvanometer had been due to forces arising outside of the thermopile they ought to continue to appear in the controls. As will be seen in figure 2, this is not the case. In this figure the top and bottom sets of tracings are the controls, the intermediate sets, that is, the second and third sets, are taken with the thermopile in circuit with the galvanometer. (For further explanation see legend to figure 1.)

In view of the fact that the signalling of the deflections of the galvanometer was not entirely automatic another precaution was taken. The drum and recording apparatus were placed in a position so that the observer of the galvanometer could not see the movements of the

FIG. 2.

heart lever. In this way the influence of suggestion or anticipation was entirely ruled out.

During the experiment from time to time the sensitivity of the galvanometer was tested with a current of 8.3×10^{-8} amperes. The sensitivity remained almost constant giving a deflection of nearly 30 mm. of scale (at 1 meter distance). The internal resistance of the galvanometer was 12.2 ohms and that of the thermopile was 14.5 ohms. As shown above these factors indicate a theoretical value of about 4.6×10^{-5}° C. for each mm. deflection. In the records (see figs. 1 and 2) each large upstroke of the galvanometer signal represents a movement through 5 mm. of scale to the right, or in the direction of positive heat production. The short upstrokes of the signal indicate a movement through 5 mm. of scale to the left or in the direction of falling temperature. Each change of temperature marked by the signal, therefore, has a theoretical value of five times the value of a 1 mm. deflection, or 2.3×10^{-4}° C.

At the end of the experiment the ventricle was freed from atria and aortae and weighed; it was found to have a weight of 3.5 grams.

The temperature of the moist-chamber was nearly 13° C., and the room temperature about 23° C. throughout the experiment.

In tables 2 and 3 the events of more than 60 heart-beats are analyzed and set down in the order in which they appear in the drum records between 1.05 and 3.10 a.m. No other selection was made than the omission of the weak atrial or partially blocked beats, and of course the beats that on account of maladjustment of apparatus or failure on the part of observer were imperfectly recorded. The time that unfortunately had to be taken out to adjust apparatus and to give periods of rest from observing galvanometer deflections, accounts for the fact that only 62 heart-beats were satisfactorily recorded out of a possible 300.

It will be noted that 0.5 second is taken as the latent period supposed to intervene between the moment of the inner stimulus and the moment when the ventricle begins to contract. This number is taken rather arbitrarily for no one can know just what the latency is in a spontaneously beating muscle. However, in observing the latency and contraction period of non-beating turtle's ventricle (3) I found that the ratio of the two periods at temperatures between 10 and 15° C. is very nearly 1:10. Since the average systole in this heart was about 4.3 seconds as shown by the tables one cannot be very far from the actual latency with the figure 0.5.

The figures in the tables that are bracketed were not included in making up the average results.

The following list gives at once an explanation of the various symbols that head the columns of figures and a summary of their average values.

θ Represents the time in seconds lapsing between the moment of beginning systole plus 0.5 seconds latency, and the moment of the rise in temperature, in other words the latency of initial temperature rise. The average value of θ, 4.56 seconds.

θ_m represents the maximum rise in temperature during the heart beat. Its average value in scale divisions is 34.2 (mm.). The average time, from beginning systole + 0.5 seconds, to reach this maximum is 7.9 seconds (θ_m in seconds required.)

Q_m represents the rate of heat production in s. d. deflection, as inferred from rate of the rise of temperature, per second of time. The average maximum rate of heat production is 12.37 s. d. (mm.) per second. The average time to reach this maximum is 6.3 seconds.

M represents the systole or the mechanical change of the ventricle in terms of mm. displacement of the writing tip of the muscle lever. The maximum shortening (M_m) has an average value of 5.43 mm. The average time to reach this maximum from the moment of beginning systole + 0.5 seconds latency is 4.8 seconds.

$M\theta$ represents the height of the muscle lever above the base line (during diastole) at the moment of maximum rise of temperature, θ_m. Its average value is 2.14 mm.

MQ_m represents the height of the muscle lever above its base line (during diastole) at the moment of the greatest rate of heat production, Q_m. Its average value is 4.18 mm.

The headings of the other columns are self-explanatory. The average duration of systole and diastole + 0.5 seconds latency, that is the cardiac cycle, appears to be 11.12 seconds. The average period of diastole alone is 6.3 seconds.

The data collected in tables 2 and 3, however, give us only the limiting values of the various factors of our problem. If we wish to know more in detail the relation of the differentials from moment to moment we may resort to the method of graphs. The questions of interest that one may answer in this way are: (1) What is the average velocity of heat production at the successive intervals during the time of its liberation? And thence (2) What is the average change of temperature from moment to moment?

To answer the questions the records of the 62 heart-beats analyzed above were plotted in toto on a sheet of squared paper here reproduced as figure 3.

The curve at the top of the figure represents the three chief phases of the heart beats, the latency to the inner stimulus, the systole and the

diastole. The vertical mm. lines of the squared paper represent each 0.05 second, the 5 mm. lines represent 0.25 second. The time relations of the three phases of the curve are the average times as found in tables 2 and 3. Along the horizontal 5 mm. lines of the paper below the

TABLE 2

NUMBER OF BEAT	θ_i IN SECONDS	θ_m IN S. D.	θ_m SECONDS REQUIRED	Q_m IN S. D.	Q_m SECONDS REQUIRED	M_m SECONDS REQUIRED
I, 1	[1.5]	30	7.5	12.0	6.5	3.8
2	5.5	15	7.5	7.0	6.5	3.5
3	4.0	35	7.0	12.0	5.0	3.8
4	4.5	20	7.0	12.0	5.0	3.5
5	4.0	20	6.8	17.0	4.7	3.5
6	4.0	25	7.5	12.0	6.0	3.7
7	5.0	15	6.0	12.0	5.5	4.5
II, 1	4.5	20	6.5	7.0	5.0	4.0
2	4.0	20	6.8	10.0	6.2	4.2
3	4.6	25	6.8	12.0	5.8	3.6
4	4.5	15	4.2	7.0	5.0	3.5
5	4.5	30	6.8	12.0	5.7	3.7
6	4.4	20	7.0	11.5	5.8	3.7
7	4.3	30	7.2	12.0	5.5	3.5
8	4.0	30	6.5	12.5	5.0	3.5
9	3.6	50	9.9	12.5	5.0	3.6
10	5.0	[10]	5.5	12.0	5.5	3.5
11	5.0	25	10.7	8.5	7.5	3.7
12	3.6	55	7.2	17.5	5.6	3.7
13	4.2	40	6.5	15.0	5.1	3.8
III, 1	4.5	25	7.5	12.0	5.0	4.0
2	3.4	35	6.5	12.5	5.3	3.8
3	4.3	40	6.8	17.5	5.8	3.8
4	4.6	40	7.7	17.5	5.8	4.3
5	4.3	45	8.0	12.5	5.4	4.0
6	5.2	35	7.9	12.5	6.5	4.5
7	2.5	55	6.5	14.0	[9.0]	5.0
8	[1.5]	30	6.0	12.0	5.5	4.0
9	5.0	25	6.8	12.5	5.6	4.1
10	2.8	25	6.8	11.0	5.2	4.5
11	3.0	40	8.4	11.0	6.0	5.0
IV, 1	5.5	30	9.0	8.0	6.5	5.0
2	(1.2?)4.2	(50) 40	7.0	12.5	5.2	4.0
3	5.0	40	8.0	15.0	6.0	5.3
4	4.7	20	8.5	10.0	6.7	5.7
5	3.0	25	6.7	11.0	5.2	5.0
6	2.8	50	7.5	18.0	5.2	4.5

TABLE 2—*Continued*

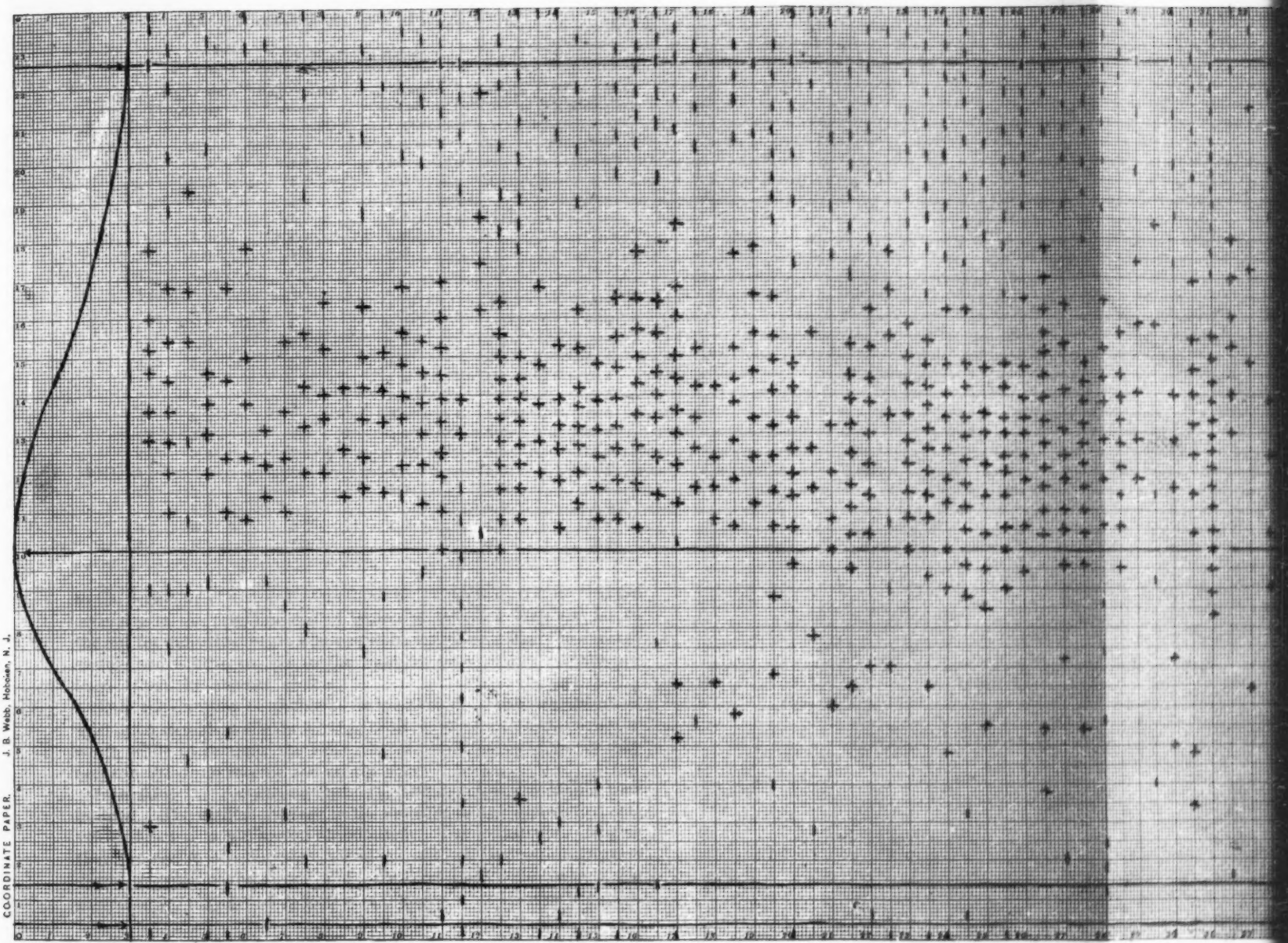
NUMBER OF BEAT	θ_i IN SECONDS	θ_m IN S. D.	θ_m SECONDS REQUIRED	Q_m IN S. D.	Q_m SECONDS REQUIRED	M_m SECONDS REQUIRED
IV, 7	3.5	45	8.5	15.0	7.2	5.2
8	7.0	20	9.2	8.5	9.0	5.5
9	5.0	50	8.5	15.0	6.3	5.0
10	4.8	55	10.0	15.0	8.2	6.5
11	2.5	50	7.8	15.0	5.8	4.8
V, 1	6.5	55	10.0	15.0	7.0	7.0
2	[2.0]	45	6.8	15.0	4.5	4.5
3	4.3	50	7.8	15.0	5.8	4.8
4	3.8	45	7.5	17.5	5.5	4.0
5	[1.5]	[80]	9.7	17.5	8.0	5.7
VI, 1	[2.0]	55	9.1	16.0	6.5	6.0
2	3.8	55	9.0	15.0	6.0	6.0
3	4.5	35	7.5	12.5	6.5	4.2
4	6.1	45	9.3	12.5	7.8	6.3
5	7.7	25	10.7	7.5	8.2	6.7
VII, 1	3.5	25	8.0	8.5	7.5	6.0
2	4.0	20	9.0	4.0	9.0	6.0
3	3.5	50	10.3	14.0	8.8	6.8
4	2.5	25	10.6	5.0	9.3	6.3
5	3.3	15	9.0	10.0	9.0	6.8
6	6.3	75	10.6	20.0	8.8	7.3
7	7.7	30	10.2	15.0	8.7	6.2
8	4.7	20	12.7	4.0	10.2	6.7
VIII, 1	5.7	20	7.5	8.0	6.2	6.0
2	7.0	35	10.3	10.0	8.3	7.5
3	3.2	20	7.2	3.5	5.5	5.5
4	7.7	20	10.5	2.6	8.5	7.2
5	7.2	25	9.5	10.0	8.0	6.5

curve are put down the deflections of the galvanometer, indicating fall of temperature with the (−) minus sign and rise of temperature with the (+) plus sign. Each sign plus or minus equals 5 mm. scale division deflection.

Obviously there will be some discrepancies plotting these changes for *individual* heart-beats along the curve of an *average* heart-beat. These discrepancies, however, may be neglected, as will soon appear, so long as the plotting approaches absolute accuracy in the region of the height of systole. It was decided, therefore, to take the vertical line extended down from the height of systole as a base, plotting the galvanometer changes to the right and left of this line in the exact time intervals to the right and left just as they appear in the drum records. In respect

TABLE 3

NUMBER OF BEAT	M_m IN MILLIMETERS	$M_{\theta m}$ IN MILLIMETERS	M_{Qm} IN MILLIMETERS	DIASTOLE IN SECONDS	WHOLE TIME OF HEART BEAT IN SECONDS + 0.5
I, 1	6.5	1.0	3.5	6.7	10.5
2	[3.5]	[0.5]	[0.8]	7.0	10.5
3	6.0	1.0	3.8	6.5	10.3
4	7.5	2.7	5.0	7.0	10.5
5	[3.0]	[0.3]	[1.5]	4.5	8.0
6	6.3	1.1	3.0	7.0	10.7
7	5.5	4.5	5.0	7.0	11.5
II, 1	5.0	2.0	4.0	7.0	11.0
2	6.5	2.5	3.5	7.0	11.2
3	6.0	2.0	3.0	7.0	10.6
4	4.0	2.0	3.0	7.0(?)	10.5(?)
5	6.5	1.7	3.0	6.5	10.2
6	6.3	2.7	3.5	6.0	9.7
7	7.0	3.5	5.0	6.0	9.5
8	6.5	2.0	4.0	6.3	9.8
9	7.0	2.0	4.5	6.0	9.6
10	7.0	4.5	4.5	6.0	9.5
11	[3.0]	[0.5]	[0.7]	7.0	10.7
12	6.0	1.0	3.5	6.5	10.2
13	6.8	2.8	5.5	6.0	9.8
III, 1	5.0	1.8	4.0	6.5	10.5
2	5.0	2.3	4.0	6.4	10.2
3	5.0	1.3	4.0	5.8	9.6
4	7.0	2.0	6.5	6.2	10.5
5	7.0	2.0	5.5	6.5	10.5
6	7.5	2.5	5.0	7.0	11.5
7	7.5	1.5	6.5	6.5	11.5
8	6.3	3.8	5.0	6.5	10.5
9	5.0	2.5	3.8	6.0	10.1
10	4.0	2.5	3.8	6.5	11.0
11	7.0	2.5	6.0	6.5	11.0
IV, 1	6.0	1.0	5.0	6.0	11.0
2	6.0	1.5	5.0	6.0	10.0
3	5.0	3.0	4.5	6.5	11.3
4	4.0	1.5	3.0	6.0	11.7
5	4.5	3.7	4.5	6.2	11.2
6	5.0	2.6	4.7	5.5	10.0
7	5.0	2.0	4.5	7.0	12.2
8	4.5	1.4	1.6	6.5	12.0
9	7.0	2.5	5.5	6.0	11.
10	6.5	1.8	4.5	7.0	13.5
11	5.0	3.0	4.3	5.0	9.8


COORDINATE PAPER. J. B. Webb, Hoboken, N. J.

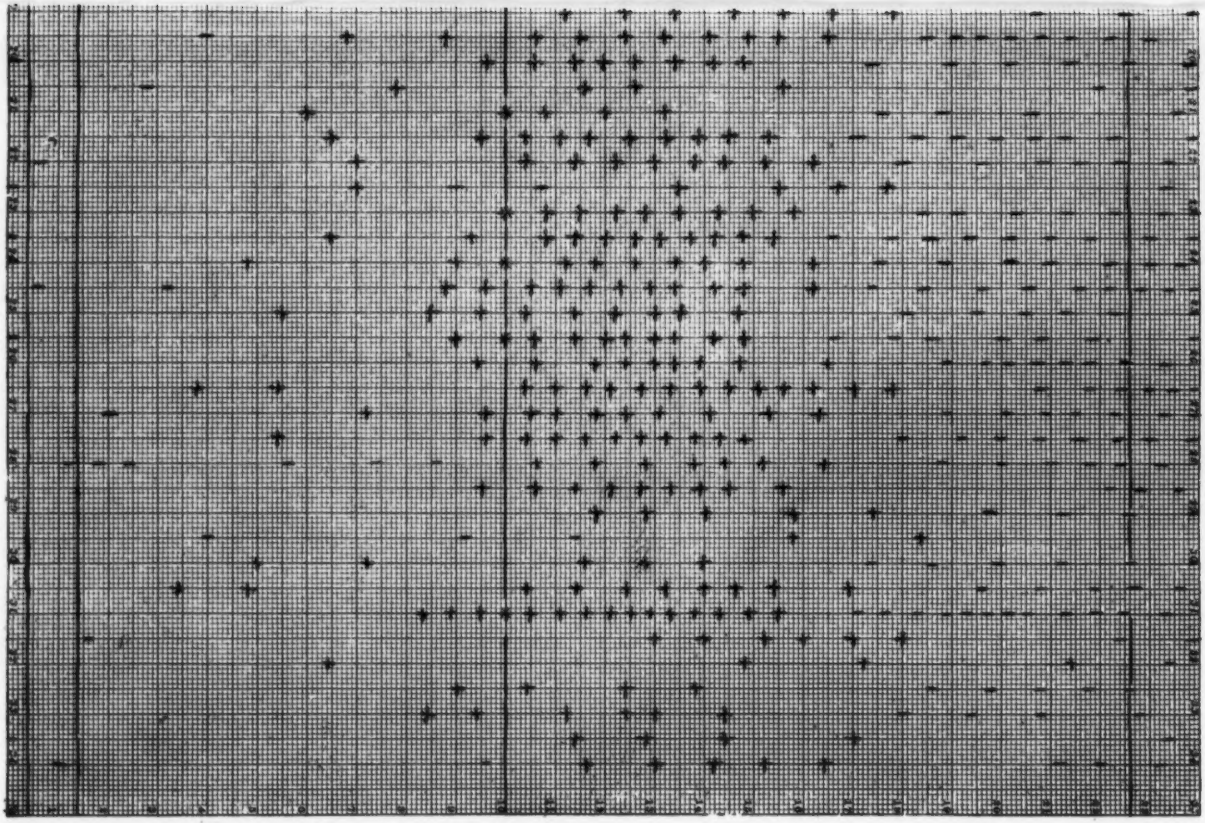


Fig. 3. Reduced to two-thirds of the original.

TABLE 3—*Continued*

NUMBER OF BEAT	M_m IN MILLIMETERS	$M_{\theta m}$ IN MILLIMETERS	M_{Qm} IN MILLIMETERS	DIASTOLE IN SECONDS	WHOLE TIME OF HEART BEAT IN SECONDS + 0.5
V, 1	3.8	1.0	3.8	5.0	12.0
2	4.3	2.3	4.3	5.5	10.0
3	3.5	2.0	3.0	4.5	9.3
4	7.0	2.0	5.5	8.0	12.0
5	8.0	2.0	5.0	7.3	13.0
VI, 1	7.0	3.2	6.8	6.0	12.0
2	6.0	4.0	6.0	5.0	11.0
3	4.0	1.0	2.5	5.2	9.5
4	4.0	3.5	4.0	5.5	11.8
5	5.0	1.5	4.5	5.5	12.2
VII, 1	2.7	1.7	2.2	4.0	10.0
2	5.0	2.5	3.5	6.0	12.0
3	5.7	1.6	4.0	5.0	11.8
4	3.0	0.6	1.3	4.5	10.8
5	3.5	2.8	2.8	6.5	13.3
6	3.5	1.0	2.8	5.0	12.3
7	6.0	2.0	4.0	6.0	12.0
8	3.8	0.8	1.7	6.5	13.2
VIII, 1	6.5	4.5	4.5	7.0	13.0
2	3.0	1.8	3.0	5.5	13.0
3	1.5	1.1	1.5	6.0	11.5
4	2.5	0.7	2.0	5.0	12.2
5	4.7	2.7	3.5	6.0	12.5

to the beginning of systole this method throws the temperature changes occurring in the neighborhood of beginning systole a little too far to the left for individual beats whose systoles are shorter than the average systole and a little too far to the right for those whose systoles are longer than the average systole.

Since the duration of systole at its greatest is 7.5 seconds and at its least is 3.5 seconds, while the average of all the 62 beats is 4.78, it may be worth while to compare the time relations of these extremes as they occur in the drum records and on the chart.

In making this comparison there are certain points one should bear in mind. The gradual increase in the duration of the systole in a surviving heart is a wellknown phenomenon. The variations so long as they proceed in one direction, therefore, can not be due in any great degree to experimental error. But, as will be seen by the controls, the galvanometer may give a deflection of 5 mm. s. d. to right or left quite

independently of what is occurring in the heart. The occasionally erratic time positions of the individual values of θ_i, θ_m and Q_m, therefore, may well be due to experimental error. Further, to any one familiar with the swinging magnet form of galvanometer it will be obvious that the probability of error will be greater for θ_i than either θ_m or Q_m.

. Again of all the points along the tracing of the muscle lever the point where one would expect least experimental error to take part is at the height of the contraction curve or at the end of systole.

The general features brought out by the chart may be put in words somewhat as follows:

As one passes along the ordinates from left to right one notes a general tendency for the minus signs to change into a series of plus signs. This denotes a change from a lower to a higher temperature in the heart muscle. The increase in general begins near the end of systole. The tendency for this initial rise to be displaced toward the beginning of systole in the records at the lower portion of the chart is due to the fact, pointed out above, that the actual duration of systole of these beats is longer than the average, 4.8 seconds, provided for by the chart. The displacement is not so great for systoles that are shorter than the average because these being more numerous are nearer the average.

It is also seen in the chart that in general the highest temperature is reached near the middle of diastole and that fall of temperature beginning in the latter half of diastole continues during the succeeding pause and latent period on into the phase of systole.

To see the detail of the changes from moment to moment one may take the averages for successive differentials of time and plot them in the form of graphs.

To do this the algebraic sums were taken of the galvanometer deflections occurring during every successive 0.25 second on the chart. The sums were then divided by the total number of heart beats (62). The final number thus obtained represents the average deflection for each successive differential of time to the right and left of the end point of systole. Since the deflections represent change of temperature and heat production our numbers represent thus the differentials of these quantities. They are collected in table 4 and appear as graphs in figure 4.

Summary of the results of the experiment of June 22, 1917. The graphs in figure 4 summarize the results obtained from the split ventricle of the terrapin at once in minuter detail and broader generalization than

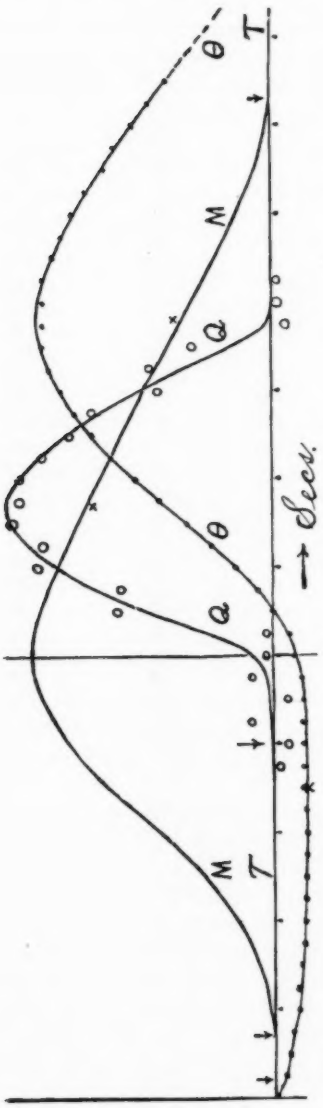


Fig. 4. This figure summarizes the results of the experiment of June 22 in the form of graphs. The ordinates connected by the curve *M M* represent the mechanical changes, those of the curve *θ θ* the temperature changes and those of the curve *Q Q* the amount of heat production occurring simultaneously at each successive moment of time. The time is represented by the abscissae in second intervals.

TABLE 4

SERIAL NUMBER OF TIME INTERVAL ($\triangle t$) 0.25 SECONDS	GALVANOMETER DEFLECTION IN S. DS. OF 5 MM. EACH ($\triangle Q$)	TOTAL TEMPERATURE CHANGE IN S. DS. OF 5 MM. EACH	SERIAL NUMBER OF TIME INTERVAL ($\triangle t$)	GALVANOMETER DEFLECTION IN S. DS. OF 5 MM. EACH ($\triangle Q$)	TOTAL TEMPERATURE CHANGE IN S. DS. OF 5 MM. EACH
1	−0.258	−0.258	25	+0.516	+1.385
2	−0.08	−0.338	26	+0.581	+1.966
3	−0.08	−0.418	27	+0.565	+2.531
4	−0.139	−0.557	28	+0.565	+3.096
5	−0.032	−0.589	29	+0.516	+3.612
6	−0.048	−0.637	30	+0.451	+4.063
7	−0.048	−0.685	31	+0.403	+4.466
8	−0.015	−0 700	32	+0.254	+4.720
9	−0.000	−0.700	33	+0.274	+4.994
10	+0.000	−0.700	34	+0.177	+5.171
11	−0.048	−0.748	35	−0.032	+5.139
12	−0.000	−0.748	36	−0.015	+5.124
13	+0.032	−0.716	37	−0.013	+5.111
14	+0.064	−0.652	38	−0.210	+4.901
15	−0.015	−0.667	39	−0.177	+4.724
16	−0.032	−0.699	40	−0.258	+4.466
17	+0.048	−0.651	41	−0.307	+4.159
18	−0.032	−0.683	42	−0.388	+3.771
19	+0.048	−0.635	43	−0.226	+3.545
20	+0.145	−0.490	44	−0.419	+3.126
21	+0.139	−0.351	45	−0.371	+2.755
22	+0.350	−0.001	46	−0.388	+2.367
23	+0.340	+0.339	47	−0.350	+2.017
24	+0.530	+0.869	48	−0.548	+1.469

is possible in written language. Therefore an explanation of the curves in this figure is all that is required to make a summary sufficiently complete.

In figure 4 the curve *M M* represents the mechanical changes of the ventricular muscle during a heart beat. The duration of the heart beat is represented by the line *T T* which is marked off into seconds. One second covers 20 of the mm. divisions of the squared paper along the (horizontal) base line. Like the usual muscle curve the ascending limb represents the shortening phase and the descending limb the relaxation phase of the contraction,—in this case the systole and diastole of the heart beat. A period of a half-second latency is marked off before the beginning of the phase of shortening. The times allotted to the shortening and relaxation phases are the average as determined from tables 2 and 3.

The curve $\theta\,\theta$ shows the change of temperature, actually the galvanometer deflections, during the average heart beat. The data for this curve are taken from table 4 (see heading "total temperature change"). It will be noted that no marked increase of temperature occurs until toward the end of systole and that the maximum of the temperature increase for the beat is observed to occur more than three seconds after the height of systole is reached, or near the middle of diastole, when the cooling of the muscle begins and continues well into the period of the next spontaneous systole.

Experiment of June 9, 1917. This was the first experiment in which the split ventricle and the insert-thermopile were used. As in the latter experiment in this one, too, the treatment resulted in a permanent partial block to the ventricle which together with the temperature (12° to 11°C.) produced a very slow ventricular rate. At first the rate was about 4 per minute but later on was less than 3 per minute. The force of the beat in this case was stronger than that of the heart of June 22. The conditions of the experiment otherwise were similar to those of the one recorded above. The sensitivity of the galvanometer was not so great, the calibrating current of one microampere giving a deflection one way of only about 160 mm. At the end of the experiment the ventricle was freed from atria and aortae and weighed; the weight was nearly 3 grams.

Upon inspecting the drum records one again notes the striking regularity of the galvanometer deflection with the changing phases of every beat of the ventricle. Of the many records taken 69 were free enough from technical error to admit of analysis and comparison. The results were plotted on a large sheet of squared paper as were those of June 22 (see fig. 3), the height of systole being taken as the point from which to measure in each case the time interval of the occurrence of each s. d. of deflection recorded. The weight of the muscle lever being allowed to hang on the heart muscle constantly, there appears in the mechanical curves a greatly prolonged contraction remainder following the chief relaxation of diastole. If one times the diastole to the end of this contraction remainder, that is, to the beginning of the next systole, the period of the "pause" approaches the vanishing point and the phases of systole and diastole make up the whole cardiac cycle. The extreme slowness of the early stages of shortening and especially of the last stages of diastole where atrial beats intervened make it difficult to pick out the absolute beginning of systole and the absolute end of diastole. This accounts in part at least for the variation of the times as found for

the two phases of the heart-beat. The temperature of the moist-chamber fell one degree during the experiment. This factor together with the usual fall in rate observed in surviving hearts, even when kept at very constant temperature, accounts for the decreasing rate observed. The durations of the systoles as charted vary from four to seven seconds, those of the diastoles vary from twelve to twenty-two seconds.

Now these wide variations would make any collective treatment of the whole number of heart beats impossible if the events we were searching for happened to occur near the beginning of systole or the end of diastole. But these results, like those described above, show in striking fashion that the chief heat production of the heart, as the Paschen galvanometer records it, begins in the latter half of systole. Thus by taking the completion of systole as the point from which to measure the times of the galvanometer deflections one has a time relation of which one can claim the greatest degree of certainty. For, as already stated, the end of systole and the beginning of diastole is a point very sharply marked on the drum records, and can be placed on the time tracer in most cases within 0.02 of a second.

The charted results of the experiment of June 9 give a picture very similar to that shown in figure 3. The algebraic sums of the temperature change for the successive seconds of time are again taken for the whole number of heart beats (69 in this case), and the averages determined. These numbers are put in the following table (table 5) in terms millimeters s. d. of galvanometer deflections. The first column indicates the order of successive time intervals in seconds, figures of the second column show the rate of heat production for each corresponding time interval, the third column consists of figures showing the whole temperature change of the heart reached at each successive interval. Plotting these data in the form of graphs, (see fig. 5) we get a picture practically the same as that shown in figure 4. Again the curve $M\ M$ represents the mechanical changes, the curve $Q\ Q$ the amount of heat production and the curve $\theta\ \theta$ the temperature of the muscle from moment to moment of the cardiac cycle. In the curves $Q\ Q$ and $\theta\ \theta$ the lines connect ordinates representing actual values erected along the abscissae of time intervals (seconds). The curve $M\ M$ is the conventional muscle curve representing the shortening and relaxation phases of the heart beat. In comparing the time relations of the other curves with the contraction curve one should use the height of the curve, i.e., the end of systole, as a point of departure, noting the number of seconds before or after the maximum point of systole that any particular event happens.

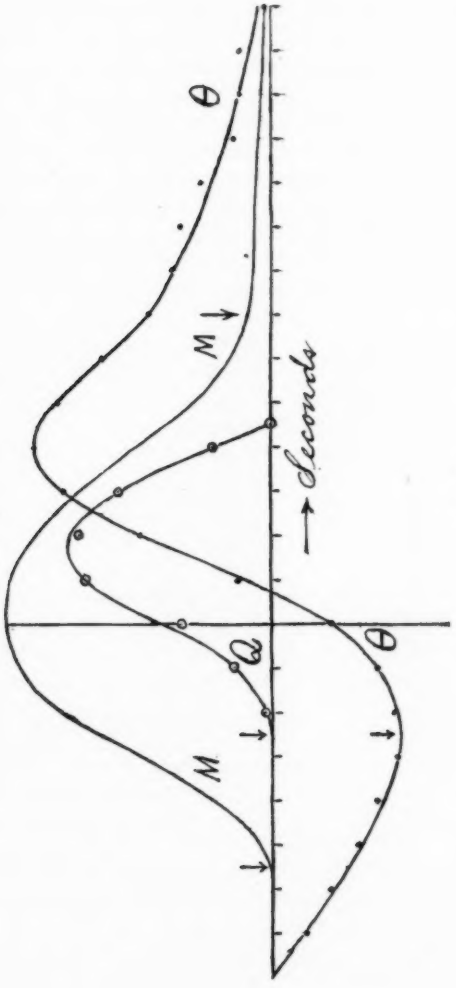


Fig. 5.

TABLE 5

ORDER OF TIME UNITS IN SECONDS ($\triangle T$)	AMOUNT OF HEAT PRODUCTION PER UNIT OF TIME IN MILLIMETER S. D. ($\triangle Q$)	TEMPERATURE OF MUSCLE AT EACH TIME INTERVAL IN MILLIMETER S. D. (θ)
1	−0.798	−0.798
2	−0.507	−1.305
3	−0.652	−1.957
4	−0.435	−2.392
5	−0.435	−2.827
6	+0.072	−2.755
7	+0.424	−2.331
8	+1.013	−1.318
9	+2.100	+0.782
10	+2.175	+2.957
11	+1.737	+4.694
12	+0.652	+5.346
13	−0.507	+4.839
14	−1.013	+3.826
15	−1.087	+2.739
16	−0.290	+2.449
17	−0.424	+2.025
18	−0.424	+1.601
19	−0.724	+0.877
20	−0.145	+0.732
21	0.000	+0.732
22	−0.870	−0.138

Experiment of May 5, 1917. In this experiment the ventricle was kept intact and the clasp-thermopile described on page 423 was used. In other respects the conditions and procedure were the same as in the experiments with the split ventricle. In adjusting the clasp-thermopile the inner sets of junctions were made to hug the ventricle walls as lightly and as yet securely as possible, not in the horizontal plane but in a diagonal plane, the hinge-side of the instrument resting in the atrio-ventricular groove and the clasp-side resting on the lower third of the ventricular wall. This disposition of the heat-junctions brought them in the closest and most constant of any position tried.

The heart chamber was kept at nearly 10°C. throughout the experiment; the beats were vigorous and regular.

The drum records show a striking rhythmicity of the galvanometer deflections synchronous with the heart-beats. Those from the middle period of the experiment were taken out in a body, and the beats analyzed and plotted as was done in the experiments reported above.

In all there were 32 heart-beats of which the following points may be noted.

The duration of systole varied from 3 to 3.75 seconds, that of diastole varied from 11.5 to 13.5 seconds. The average rate thus was nearly 4 per minute. The chart of the temperature changes in its general appearance is very like the charts of the experiments of June 9 and 22. Here again the deflections of the galvanometer indicating increments of temperature for the most part fall just in the beginning of diastole, while those indicating decrements of temperature fall chiefly in the latter part of diastole and, to a lesser extent, in early systole.

Taking algebraic sums and averages of the charted results one gets values for Q and θ for the successive intervals of time as shown on table 6, which when plotted on coördinate paper give graphs as shown in figure 6.

TABLE 6

SERIAL TIME INTERVAL IN SECONDS	Q IN MILLIMETERS S. D.	θ IN MILLIMETERS S. D.
1	−0.625	−0.625
2	−0.625	−1.25
3	−1.87	−3.12
4	−0.93	−4.05
5	+2.81	−1.24
6	+4.68	+3.43
7	+4.68	+8.11
8	+5.63	+13.74
9	−0.94	+12.80
10	−1.25	+11.55
11	−4.68	+6.87
12	−3.12	+3.75
13	−1.87	+1.88
14	−0.63	+1.25
15	−1.87	−0.62
16	0.000	−0.62
17	−0.312	−0.93
18	−0.625	−1.55

Upon comparing the graphs of figure 6 with those of figure 4 and 5 one notes a greater abruptness in the heat curves of the former. In my opinion this indicates that the clasp-thermopile did not press as evenly against the heart muscle at all times during a beat as did the insert-thermopile. In their general tendencies, however, there is a surprisingly great similarity between the results obtained from the two thermopiles.

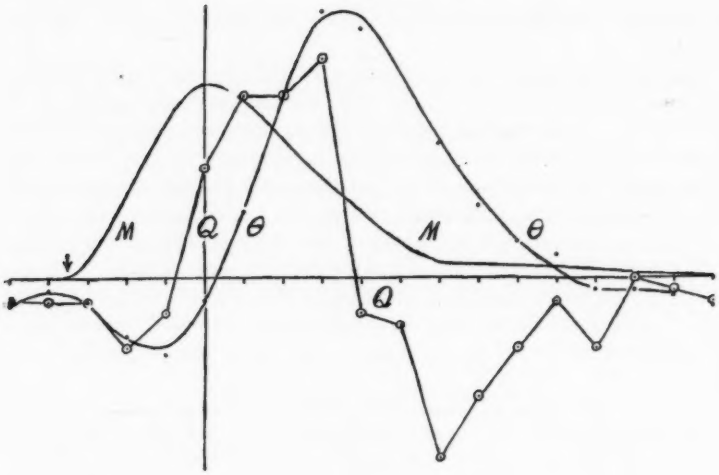


Fig. 6.

SUMMARY AND DISCUSSION OF RESULTS

A summary and discussion of the results of the present investigation may best be done under two separate headings: (1) The time relations of the chief events of heat-production in respect to the major mechanical events of the cardiac cycle, and (2) The amount of heat produced during a heart-beat and the relation of the same to the metabolism of the heart.

1. In the early part of this study it was supposed that the latent period of contraction would prove to be a period of time that one could not well omit from consideration. From the curves it is clear, however, now that no heat is observed during that interval of time. In summarizing the durations of times of the chief events of heat production it will be best for the present, then, to use the initial rise of the muscle lever, that is initial muscle shortening (M_i), as a point from which to make the measurement. The times then from this point to maximum contraction (M_m), to the initial rise of temperature (θ_i), to the point of maximum rate in heat production (Q_m), and to the moment when the temperature of the muscle is highest for the whole beat (θ_m), may be tabulated as they appear in the three different experiments. The data are taken from the graphs in figures 4, 5 and 6.

TABLE 7

DATE OF EXPERIMENT	M_m IN SECONDS	θ_i IN SECONDS	Q_m IN SECONDS	θ_m IN SECONDS
June 22	4.3	2.8	6.0	8.1
June 9	5.5	3.0	7.25	9.5
May 5	3.5	2.3	(5.5)*	7.1

* The number in the unsmoothed curve is 6.5, but obviously it is more nearly 5.5 if the curve were smooth.

The figures in the table (7) refer to observations. Can we now make a statement as to the moment when the heat production in reality begins in the heart? When does it reach its greatest intensity and when does it end?

To answer these questions some of the corrections necessary to be made have already been referred to, namely, the delays of the recording apparatus. From what has been said on this point and in consideration of the long periods shown in the table in comparison with the few hundredths of a second those delays amount to, it is quite safe to neglect them. The delays represented by conduction of heat through the substances involved may be of greater moment. There are certain considerations, however, that make it improbable that any large fraction of the times tabulated above is taken up by the conduction of heat. The fact that all the muscle fibers are thrown into activity nearly at the same time and to the same extent would lead one to suppose that the heat production also would be nearly uniform throughout the muscle mass and therefore the rise of temperature in one part synchronous with that of another part. There would thus be no considerable distance for the heat to be conducted save the few microns represented by the serous coverings of the ventricle, or in the case of the split ventricle, the few microns represented by the fibers damaged by the section. The greatest delay in conduction would be that required to pass through the pellicle covering the wires of the thermopile and this as is well known is perhaps not more than 0.01 mm. in thickness. Bernstein (2), 1914, has discussed this problem and treated it mathematically. He has only been able to give it expression in an integral equation of indefinite form, which permits of no further solution.

Of all the factors that go to make up the delays in the deflection of galvanometer that of the instrument's period of oscillation plays by far the greatest rôle. As already stated, this is about two seconds to a current from an electric battery, that is to a current that reaches its

maximum intensity instantly. What would the period be to a current that only reaches its greatest intensity gradually as we may well imagine the thermoelectric current produced by the muscle really developes? If the current at first is very small and grows rapidly in intensity during the period of contraction, its velocity of development being greatest at the end of contraction, and if it is only the intensity at this later stage that is great enough to affect the galvanometer strongly, then we may expect, counting from the moment of this greatest intensity, a period nearly equal to that produced by a battery current. One ought also to have in mind the causes of the delay to the initial deflection of the galvanometer. If the current is like a battery current and reaches its maximum intensity practically instantly, then the delay in the initial response for the Paschen is so small as to be negligible. If on the other hand the current strength is at first exceedingly small and grows with increasing velocity only at the end of a certain period then the delay in the initial response of the galvanometer may be very great. Again it must be remembered that the initial deflection recorded in the experiments was not the actual initial deflection, obviously that would be impossible, but the deflection to a division of scale that was considered quite safe to register as such. For this reason the data from the smoothed curves in the figures will be more reliable for consideration at this time than the numerical data in the tables of the individual experiments.

With all the precautions that one should bear in mind in the interpretation of the results thus set forth, we may now proceed to the answer of our questions.

Referring to table 7 one notes that the delay between initial mechanical shortening and initial rise of temperature is more than half the duration of mechanical shortening, i.e., in the three experiments:

$$M_m = 4.3,\ 5.5 \text{ and } 3.5, \text{ respectively, while}$$
$$\theta_i = 2.8,\ 3.0 \text{ and } 2.3, \text{ respectively;}$$

whence $$\frac{\theta_i}{M_m} = .65,\ .55 \text{ and } .66, \text{ respectively.}$$

The significance of this ratio is twofold. Insofar as it approaches a constant it is fundamental evidence that the thermo-electric current, hence the heat production, is intimately associated with the process of developing contraction. The magnitude of the constant would indicate that if any heat is produced in the early stages of muscle shortening it is too small to be looked upon as the cause of the contraction, (the

heat curve as plotted at θ_i could not be given a thermal value greater than 4.6×10^{-6}°C.) but as a concomitant process that follows rather than precedes the mechanical process.

It was stated that in case the intensity of current were very small and developed relatively slowly the delay in the period of the galvanometer may be prolonged beyond that in response to closure of a battery current. The duration between θ_m and θ_i (table 7) is 5.3, 6.5 and 4.8 seconds respectively, exceeding the period of the galvanometer from two to three times. The intensity of the muscle's thermoelectric current very clearly developes slowly.

The position of Q_m in all cases is nearer θ_m than θ_i, i.e., $\theta_m - Q_m$ equals 2.1, 2.25 and (1.6) seconds whereas $Q_m - \theta_i$ equals 3.2, 4.25 and (3.2) seconds. From this as well as directly from the graphs themselves, it is evident that the heat production reaches its maximum velocity per unit of time nearer the end than the beginning of the process.

Passing now to a consideration of the time required to reach the moment of greatest velocity of heat production, Q_m, one notes at once that it is greater than the time of the mechanical process, M_m itself, and further that it is greater by 1.7, 1.75 and (2.0) seconds in the three experiments.

It may be stated again that where a small current developes relatively slowly and reaches a sufficient intensity to produce marked deflection of the galvanometer only after a marked delay, then the deflection to this maximum intensity may itself have a period approximating that in response to an adequate battery current.

The fact that this difference between Q_m and M_m is so nearly two seconds I take to be evidence that the heat production increases rapidly in intensity just before the maximum point of contraction is reached, and the thermoelectric current thus produced, acting like a battery current suddenly thrown in circuit, is responded to by the galvanometer with its usual period.

From the foregoing experiments *it may thus be concluded that the heat production of the terrapin's ventricle during spontaneous contraction begins with, or shortly after, the beginning shortening of the muscle, reaches its greatest velocity of output at, or just before, the height of contraction and then continues in lessening amount during diastole for a period of time equal to about half of the time of the preceding contraction.*

These conclusions are in accord with those of Blix (4) in his work on skeletal muscle in which it was shown that heat production occurs only where there is actual contraction, that with minimal contraction

there is also minimal heat production and with maximal contraction maximal heat production.

In view of Blix's work it would be more probable that the great delay in θ_i, as observed above, is not evidence that no heat is evolved in the earliest stages of muscle shortening but rather that the amount is so small as not to be observable.

The fact that after Q_m is reached heat is not observed to be evolved for a period more than half of that of M_m may be explained by the view that the contraction of a spontaneously beating heart is not a tetanus but a simple twitch in response to a single spontaneous stimulus.

2. The quantity of heat evolved or the amount of temperature change in the beating heart, and the metabolism thereby represented, may now be taken up.

The values of θ_m and Q_m as represented in millimeters s. d. of galvanometer deflections in the graphs (figs. 4, 5 and 6) are as follows:

DATE OF EXPERIMENT	θ_m MILLIMETERS S. D.	Q_m MILLIMETER S. D.	$\frac{Q_m}{\theta_m}$
June 22	29.3	11.1	0.38
June 9	8.2	2.3	0.28
May 5	17.8	5.4	0.30

That the values of θ_m and Q_m in the individual experiments vary so greatly is due to the fact that the galvanometer's sensitivity was different in the experiments.

The ratio Q_m/θ_m indicates that about one-third of the total rise in temperature occurs in a time interval of about one-fifth of the whole period of rising temperature $(\theta_m - \theta_i)$, or a little more than one-fifth of the whole period of mechanical change (M_m). That this ratio is roughly a constant for the three experiments may be regarded as additional evidence that the processes observed are intrinsic phenomena of the act of contraction.

Passing now to the values of the galvanometer deflections in terms of degrees temperature, as already pointed out, only the theoretical electromotive force of the thermopiles used can be employed in this discussion as a basis of calculation.

From the data and formula given on page 424, θ_m for the experiment of June 22 has a value of

$$29.3 \times 4.62 \times 10^{-5} = 1.35 \times 10^{-3}°\text{C}.$$

From the data for the experiment of June 9 (see page 442) the galvanometer's sensitivity was,

$$\Delta t = \frac{14.5 + 12.2}{30 \cdot 53 \cdot 10^{-6} \cdot 150} \cdot 10^{-6}$$

hence

$$\Delta t = 1.12 \times 10^{-4}, \text{ and}$$
$$\theta_m = 8.2 \times \Delta t = 9.2 \times 10^{-4} \text{ °C.}$$

For May 5 the sensitivity of the galvanometer to the calibrating current of 1 micro ampere was about 220 mm. s. d. The resistance of the clasp thermopile was nearly 11.32 ohms. Hence

$$\theta_m = \frac{11.32 + 12.2}{30 \cdot 53 \cdot 10^{-6} \cdot 220} \cdot 10^{-6} \cdot 17.8 = 1.2 \times 10^{-3} \text{ °C.}$$

To calculate the amount of heat in gram calories that the ventricle has to produce per gram of muscle to raise the temperature to the values thus found, one assumes that no heat was lost by radiation or conduction to the exterior during the time of observation. Taking Rosenthal's determination of the sp. ht. of cross-striped muscle, 0.825, to be about that of terrapin's ventricle and knowing the weight of ventricle[1] in each case, one proceeds in the usual manner.

$$\text{Calories heat per gram} = \frac{\theta}{0.825} \cdot \frac{1}{\text{wt.}}$$

Substituting the data of the experiment of June 22, we have

$$\frac{1.32 \times 10^{-3}}{0.825 \times 3.1} = 4.8 \times 10^{-4} \text{ calories.}$$

Substituting the data for June 9:

$$\frac{9.2 \times 10^{-4}}{0.825 \times 2.7} = 4.1 \times 10^{-4} \text{ calories.}$$

[1] The number taken for the weight in each case was reduced for the split ventricle to 0.1 less than the actual weight, on the supposition that about that amount of the tissue was rendered inactive by the process of splitting. The heart for the experiment of May 5 was not split, but unfortunately there is no record of the weight of the ventricle. The full weights of the other two ventricles were 3.5 and 3.0, and the terrapins used at that period were all very nearly the same size. The weight 3.3 put in the formula is a mere approximation, but one cannot believe it to be very erroneous, that is not more so than the guess as to the percentage of tissue destroyed by the splitting in the other two cases.

Substituting the data for May 5:

$$\frac{1.2 \times 10^{-3}}{0.825 \times 3.35} = 4.3 \times 10^{-4} \text{ calories.}$$

The number for the experiment of June 22 is based upon data that in my judgment are more accurate than those of the other two experiments. So in making a final statement of the heat production of heart muscle per beat let us rather choose the result of that experiment.

If the heat produced by the heart has its source in the oxidation of dextrose the weight of that substance oxidized per beat per gram of terrapin's ventricle, using Stohmann's heat of combustion for dextrose, is

$$\frac{4.8 \times 10^{-4}}{3688}, \text{ or } 1.3 \times 10^{-7} \text{ grams.}$$

Given the rate of beat and the output of heat, the oxidation of dextrose per gram of ventricle per hour may be calculated. The average duration of beat in the terrapin's heart for the experiment of June 22 was 11.1; in one hour there would then be 324.5 beats, the heat of which would equal 1.55×10^{-1} calories, requiring a combustion of 4.2×10^{-5} gram dextrose.

One may conclude then that the ventricle of the terrapin per gram weight and per hour at the rate of 5.4 beats per minute produces nearly 0.155 calories of heat or oxidizes the equivalent of 0.042 mgm. of dextrose.

Finally it may be pointed out that these figures compare favorably with observations on the metabolism of the heart.

The output of CO_2 per hour has been observed by Vernon (5) in the surviving tortoise heart. Taking the observations when the heart's rate was 5.5 per minute, that is about the same as it was in my experiment, one finds the output to be 41 to 47 cc. per hour per kilogram of heart (*loc. cit.*, p. 297). This volume of CO_2 is equivalent to about 0.054 mgm. dextrose and 0.187 calories of heat per gram of heart per hour, a gratifying agreement with the results of this investigation.

The results obtained from the direct determination of dextrose utilized by the surviving mammalian heart agree almost as well. In one of the most careful researches yet done on this problem Admont Clarke (6) observed, on surviving dogs' hearts perfused with oxygenated Locke's solution, an utilization from 0.013 to 0.415 mgm. of dex-

trose per gram heart per hour.[2] The highest figure is about ten times greater than that for the terrapin's ventricle; but could corrections be made for temperature, this difference doubtless would disappear.

On the other hand from Herlitzka's work (1) from 5×10^{-6} to $14.\times10^{-6}$ calories of heat is liberated per gram of rabbit's heart per beat. The equivalent of these figures in terms of dextrose is 1.3×10^{-9} to 3.8×10^{-9} gram. From these figures I estimate the dextrose consumption per hour could be no more than 0.026 mgm.

The fact that this figure is smaller than my estimate for the terrapin's heart, using the split ventricle, than Vernon's from his CO_2 determinations, and than Clarke's on direct determination of dextrose utilization, indicates that all the heat could not have been measured by the methods used in the experiments on the rabbit's heart.

CONCLUSIONS

A special method is employed in the effort to make accurate observations on the heat liberated by the beating heart. By dividing the ventricle of the surviving organ one is able to insert a sensitive thermopile and insure a greater constancy of contact between junctions and tissue than is possible with the "umfassende" or clasp thermopile.

Upon decreasing the rate of beat of such hearts by lowering the temperature or establishing block, or both, one may use a galvanometer for the observation of changing temperature whose period is far less rapid than that of the thread galvanometer, if only its sensitivity is greater. The Paschen galvanometer, which meets these requirements, was therefore the instrument selected for the experiments.

Careful analysis and reconstruction of the results obtained from a large number of beats in three different experiments lead to the following conclusions in respect to the ventricle of the terrapin's heart.

1. The heat liberated during the beat of the ventricle for the most part is liberated in the second half of systole. *a.* The liberation of heat during the first half of systole is very small and develops its velocity of output only gradually, that is, only as an exponential function of the time. *b.* The initial liberation of heat, *a priori*, being infinitely small, could not be observed. Judging however from the major part of the curve of velocity of heat production which could

[2] With the pancreas perfused in series with the heart the utilization of dextrose was greater. The results obtained by perfusing the heart alone are taken as being more comparable to the conditions of the present investigation.

be observed, it is concluded that no heat whatever is produced before actual contraction of the muscle begins. *c.* The absence of prolonged heat production during the cardiac cycle is explained by the fact that the heart beat resembles a muscle twitch rather than a tetanic response to the inner stimulus.

2. *a.* The average rise and fall in temperature of the heart at each beat is nearly 0.0013°C. The average amount of heat liberated per beat and per gram of ventricle is about 0.00048 small calories. Reckoning with the rate observed during the experiments this amounts to nearly 0.155 calories per gram ventricle per hour. *b.* If the source of this heat is in the oxidation of dextrose (or a carbohydrate of similar percentage composition) one gram of ventricle must then utilize 4.2×10^{-5} gram of the substance. *c.* Since the quantity of dextrose is nearly the amount demanded (5.4×10^{-5} gram) by the CO_2 output of tortoise's heart as observed by Vernon under similar conditions and is nearly the amount, if corrected for temperature, etc., as was measured directly by Admont Clarke on surviving dogs' hearts, it is concluded that dextrose or some carbohydrate of equal heat of combustion is the source of the heat liberated by the beating heart.

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